Phase I/II study augmenting TAK-659 action in relapsed/refractory AML by addition of the proteasome inhibitor Ixazomib
Big Ten Cancer Research Consortium BTCRC-HEM17-092

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PROTOCOL SIGNATURE PAGE

Phase I/II study augmenting TAK-659 action in relapsed/refractory AML
by addition of the proteasome inhibitor Ixazomib

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SYNOPSIS

TITLE	Phase I/II study augmenting TAK-659 action in relapsed/refractory AML by addition of the proteasome inhibitor Ixazomib
PHASE	Phase I/II
OBJECTIVES	Primary Objective: **Phase I**: to determine the safety, tolerability, and maximum tolerated dose (MTD), recommended phase 2 dose(s) (RP2D) of the combination of TAK-659 and Ixazomib administered orally on a daily and weekly dosing schedule, respectively, in patients with relapsed or refractory AML **Phase II**: to evaluate preliminary efficacy of TAK-659 plus Ixazomib in relapsed or refractory AML as measured by overall response rate (ORR) Secondary Objectives: • To evaluate the differential efficacy measures of TAK-659 with Ixazomib, involving presence or absence of Flt3 mutation(s), and/or mutational profile by NGS, additionally affecting epigenetic modifiers • To evaluate additional efficacy measures of TAK-659 with Ixazomib, such as duration of response (DOR), time to progression (TTP), mortality rate at 3 and 6 months, and overall survival (OS) • To evaluate the influence of multiple cycles of the combination on the outcome measures when applied in context of subsequent feasibility for bridge to transplantation as consolidation therapy Exploratory Objectives: • To quantitatively examine before, and at early timepoint of therapy, the primary mechanistic transcriptional drivers of these AMLs (HOXA and MEIS1), which are postulated to be extinguished by the drug combination, for their ability, when extinguished, to predict subsequent response • To implicate Syk, Wnt pathway (ID1, JUN, FOXM1, β-catenin) and Wnt antagonist RUNX3 in responses affected by combination of TAK-659 and Ixazomib • To use digital PCR technology to examine the participation of collaborating mutations on depth of remission for the class 1 and class 2 drivers
STUDY DESIGN	This study will include a phase I dose finding portion, and a single-arm phase II portion in relapsed or refractory AML, for use of TAK-659 and Ixazomib in combination. The starting TAK-659 dose for the phase I portion of the combination trial will use a dose below RP2D dose of TAK-659 established in C34002, 100 mg po once daily. If 2 DLTs are recorded in the second cohort of this 3+3 dose-tier of C34002, then a dose of 80mg in single dose administration will be recommended. The TAK-659 treatment duration in the combination trial will be days 1-15 of a 21day cycle (as compared to a 28 day course of single agent administration in C34002).

	For the addition of Ixazomib into the combination, we will adopt the experience for single agent Ixazomib use, which revealed similar pharmacokinetics (AUC 0-168h) and 2 or 3-fold accumulation during a cycle for weekly and twice-weekly schedules, respectively (the MTD for Ixazomib dosing given alone was 2mg/m^2 twice weekly d1, 4, 8, 11 of a 21 day cycle, whereas once weekly dosing given alone was at 2.97 mg/m^2 on days 1, 8, 15 of a 28 day cycle). Thus, when initiating a dose-finding escalation, in a schedule with TAK-659, significant dose reduction from the single agent Ixazomib RP2D weekly 4mg amount may not require more than two dose-steps, thus resulting in a plan for Ixazomib 2.3mg po day 1,8, 15 of the 21 day cycle. From this dose, escalation will be undertaken. Given the expected toxicity associated with Ixazomib, where diarrhea or neuropathy have previously been implicated, if 2/6 patients were to develop such ≥ grade 3 nonhematologic toxicity at the +1 dose of 3mg, then the next cohort would receive reduced dosing at 2.3mg po days 1,8,15. However, if increased amylase and/or AST elevations were to occur, implicating TAK-659 as culprit, then one-step dose reduction of that agent would provide direction for further pursuit of the algorithm. Dose escalation will continue until either MTD is reached or the RP2D (if different than MTD) has been determined based on safety, tolerability, PD, and preliminary efficacy data. The culmination of the 3+3 algorithm would be an expected MTD involving 15 days administration of derived daily dose for TAK-659, along with derived weekly dose for Ixazomib on days 1, 8, 15. The process for determining or refining the RP2D, will allow expansion of more than 1 dose/schedule level to at least 6 patients (up to a maximum of 12 evaluable patients per dose/schedule level is permissible) so that pharmacodynamic measures and early signs of clinical activity can be assessed. This is important in a disease process as AML, for which some immediate level of required drug-induced myelosuppression during blast clearance, will defer endpoint recognition. The sample sizes for the Phase II study are estimated using a one-sided test at a significance level of α= 0.1 with power of 80%. To perform the phase 2 study, we will consider a Simon's 2-stage design (See Study Overview Diagram). The primary objective of the phase 2 portion of the study is to detect an efficacy signal that warrants further development of the combination in AML, which will likely be annotated by molecular genetic mutational subgroups. It is most likely those will exist within the rubric of Flt3 mutation. The primary measure of efficacy for the phase 2 portion will be ORR, which will include CR, CRp, CRi, and PR. Best response will be assessed by the end of Cycle 3 of TAK-659/Ixazomib combination for the purpose of an interim analysis between Stage 1 and Stage 2. The Flt3WT and Flt3mut cohorts will proceed to the second stage if patients respond by CR, CRp, CRi. The Flt3WT cohort uses a null hypothesis response rate of ≤ 15%, versus an alternative hypothesis of response rate ≥ 40%. Based on a Simon 2-stage Minimax design, approximately 9 patients will be accrued in

	the first stage. If there are 1 or fewer responses in the 9 patients, the study will be stopped. Otherwise, 7 additional patients will be accrued for a total of 16. The treatment will be recommended for further study if 5 or more responses are observed in 16 patients The Flt3 mutant cohort uses as null hypothesis of response rate ≤30%, versus an alternative hypothesis of response rate ≥ 55%. Based on a Simon 2-stage Optimal design, 8 patients will be accrued in the first stage. If there are 2 or fewer responses in the 8 patients, the study will be stopped. Otherwise, 12 additional patients will be accrued for a total of 20. The treatment will be recommended for further study if 8 or more responses are observed in 20 patients. Other efficacy measures, such as DOR, TTP, ability of the therapy to provide bridge to transplant, and mortality rate will also be considered in the decision to expand the study. Best responses from both cohorts will be assessed individually and combined in an effort to understand the all-comer response rate. Retrospective analysis will be performed to identify potential patient selection markers for response, including the expressions of Syk, and the Wnt pathway agonists (ID1, JUN, FOXM1, β-catenin) and antagonist RUNX3, and the end-targets expected of TAK-659 and Ixazomib, HOXA and MEIS1, when measured before and then sampled early into therapy. Interpretation of these measures will be made in context of mutational profile of cooperating epigenetic mutations, especially involving TET2, IDH2/1, WT1, as well as DNMT3A, without or with Flt3 mutation, available by next-generation sequencing of patient marrow samples.
KEY ELIGIBILITY CRITERIA	Patients will be males and females age 18 years or older with histopathologically documented primary or secondary AML as defined by World Health Organization, other than acute promyelocytic leukemia, or complex karyotype or monosomy 7 (or containing monosomy 7), with relapsed or refractory disease for whom no standard therapies are anticipated to result in durable remission according to the clinical judgment of the treating physician, or who refuse standard therapies. (See body of protocol for full criteria.) These AML's will have been further phenotyped genetically to include normal karyotype, tMLL, or nonrecurring mutation(s). For the phase 2 portion of the study, relapsed/refractory patients must be refractory to or relapsed after no more than 2 prior chemotherapy regimens and must not have prior exposure to any investigational Flt3 inhibitors (midostaurin and gilteritinib are allowed). Patients must have adequate organ function and an Eastern Cooperative Group (ECOG) performance status of 0 to 2. Patients must not have clinically significant toxicity from prior chemotherapy, hematopoietic stem cell transplant (HSCT) within 60 days of the first dose of TAK-659/Ixazomib, or clinically significant graft-versus-host disease requiring ongoing immunosuppressive therapy.

STATISTICAL CONSIDERATIONS	During the dose escalation phase, dose escalation will be conducted according to a standard 3+3 dose escalation schema, and up to 18 response-evaluable patients will be enrolled. The MTD/RP2D cohort will have at least 6 patients. The sample sizes for the Phase II study are estimated using a one-sided test at a significance level of α= 0.1 with power of 80%. To perform the phase 2 study, we will consider a Simon's stage 2 design (See Study Overview Diagram). The primary objective of the phase 2 portion of the study is to detect an efficacy signal that warrants further development of the combination in AML, which will likely be annotated by molecular genetic mutational subgroups. It is most likely those will exist within the rubric of Flt3 mutation. The primary measure of efficacy for the phase 2 portion will be ORR, which will include CR, CRp, CRi, and PR. Best response will be assessed by the end of Cycle 3 of TAK-659/Ixazomib combination for the purpose of an interim analysis between Stage 1 and Stage 2. The Flt3WT and Flt3mut cohorts will proceed to the second stage if patients respond by CR, CRp, CRi. The Flt3WT cohort uses a null hypothesis response rate of ≤ 15%, versus an alternative hypothesis of response rate ≥ 40%. Based on a Simon 2-stage Minimax design, approximately 9 patients will be accrued in the first stage. If there are 1 or fewer responses in the 9 patients, the study will be stopped. Otherwise, 7 additional patients will be accrued for a total of 16. The treatment will be recommended for further study if 5 or more responses are observed in 16 patients The Flt3 mutant cohort uses as null hypothesis of response rate ≤30%, versus an alternative hypothesis of response rate ≥ 55%. Based on a Simon 2-stage Optimal design, 8 patients will be accrued in the first stage. If there are 2 or fewer responses in the 8 patients, the study will be stopped. Otherwise, 12 additional patients will be accrued for a total of 20. The treatment will be recommended for further study if 8 or more responses are observed in 20 patients. Other efficacy measures, such as DOR, TTP, ability of the therapy to provide bridge to transplant, and mortality rate will also be considered in the decision to expand the study. Best responses from both cohorts will be assessed individually and combined in an effort to understand the all-comer response rate.
TOTAL NUMBER OF SUBJECTS	N = 54 Phase I= up to 18; Phase II= up to 36 (16+20)
ESTIMATED ENROLLMENT PERIOD	24-32 months, including 9-12 months in the phase I portion and 15-20 months in the phase 2 portion
ESTIMATED STUDY DURATION	48 months

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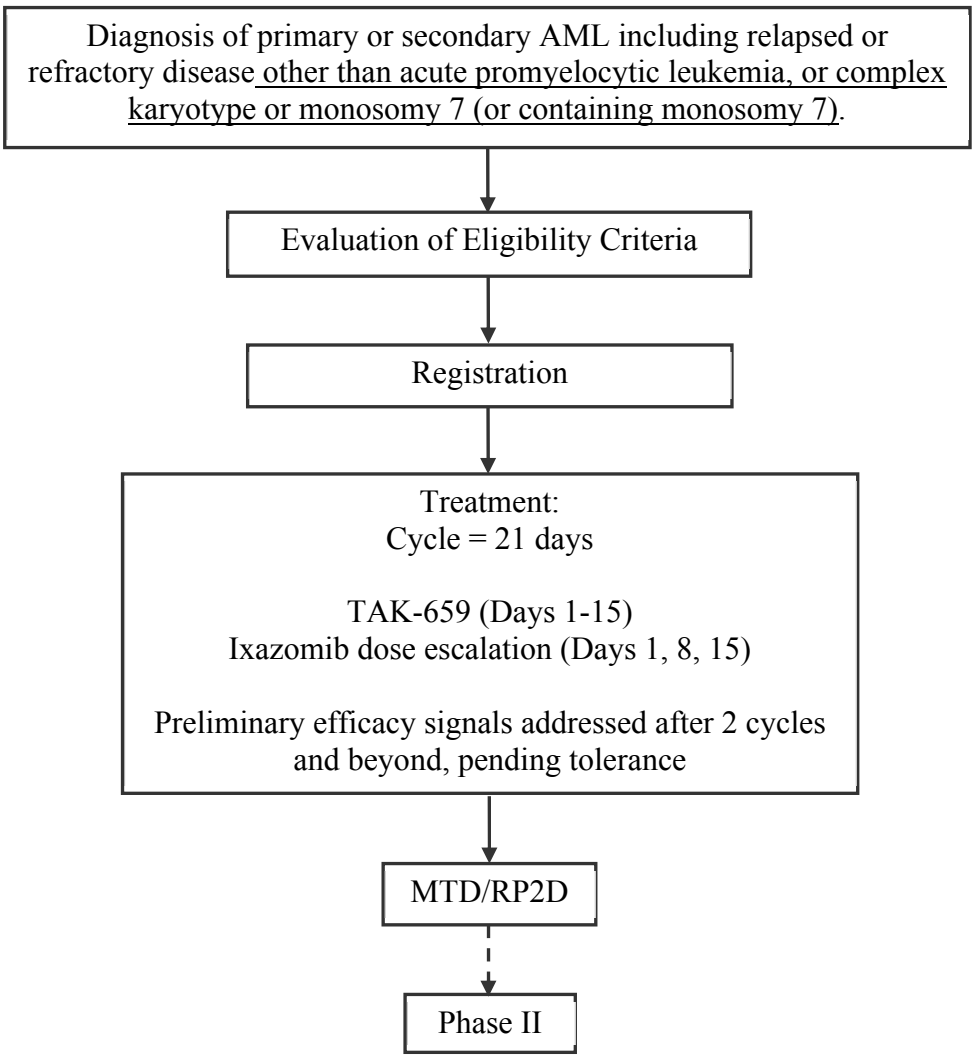
PHASE I SCHEMA



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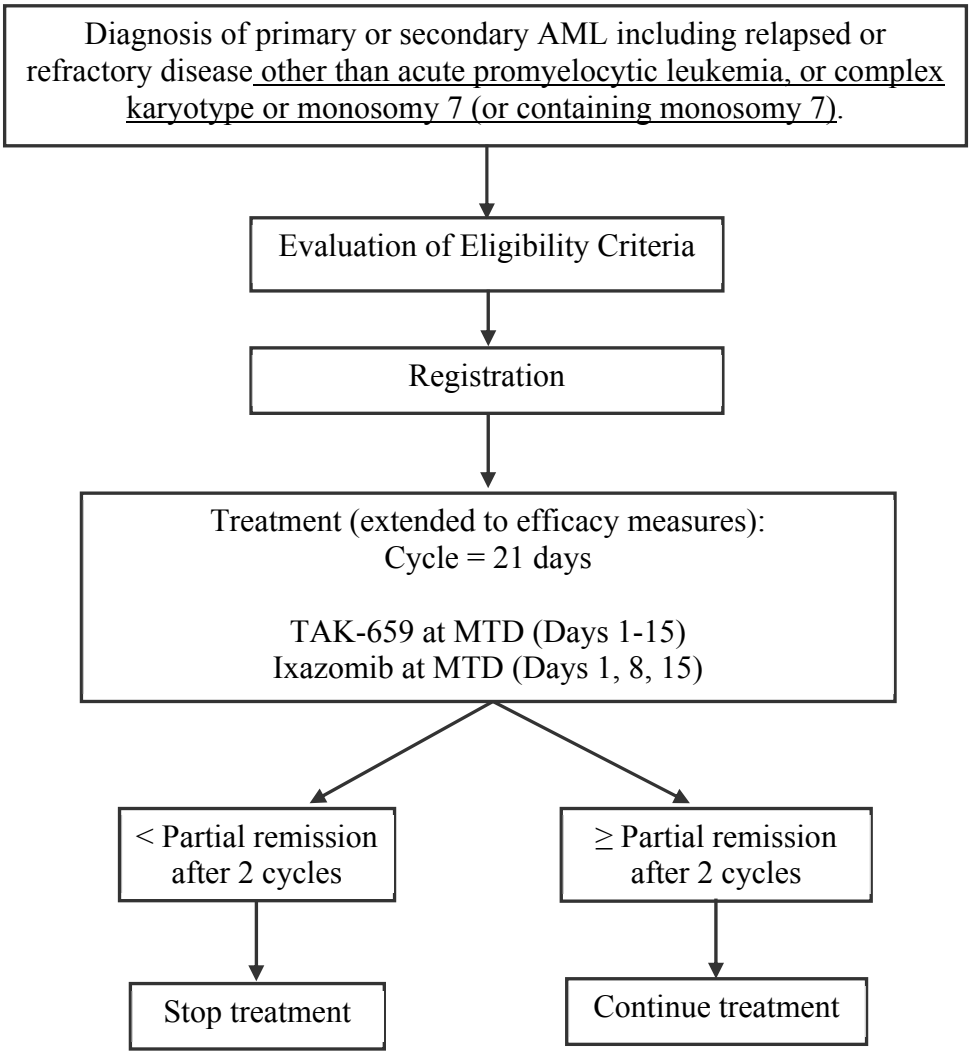
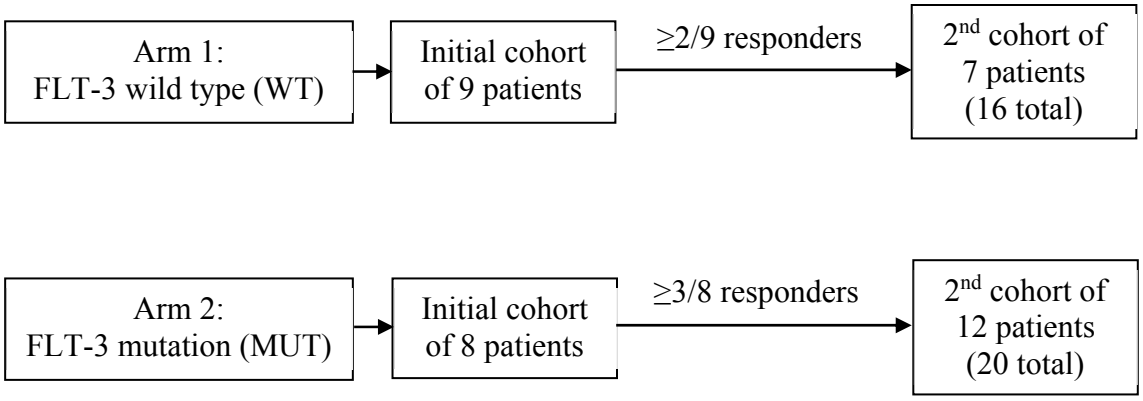
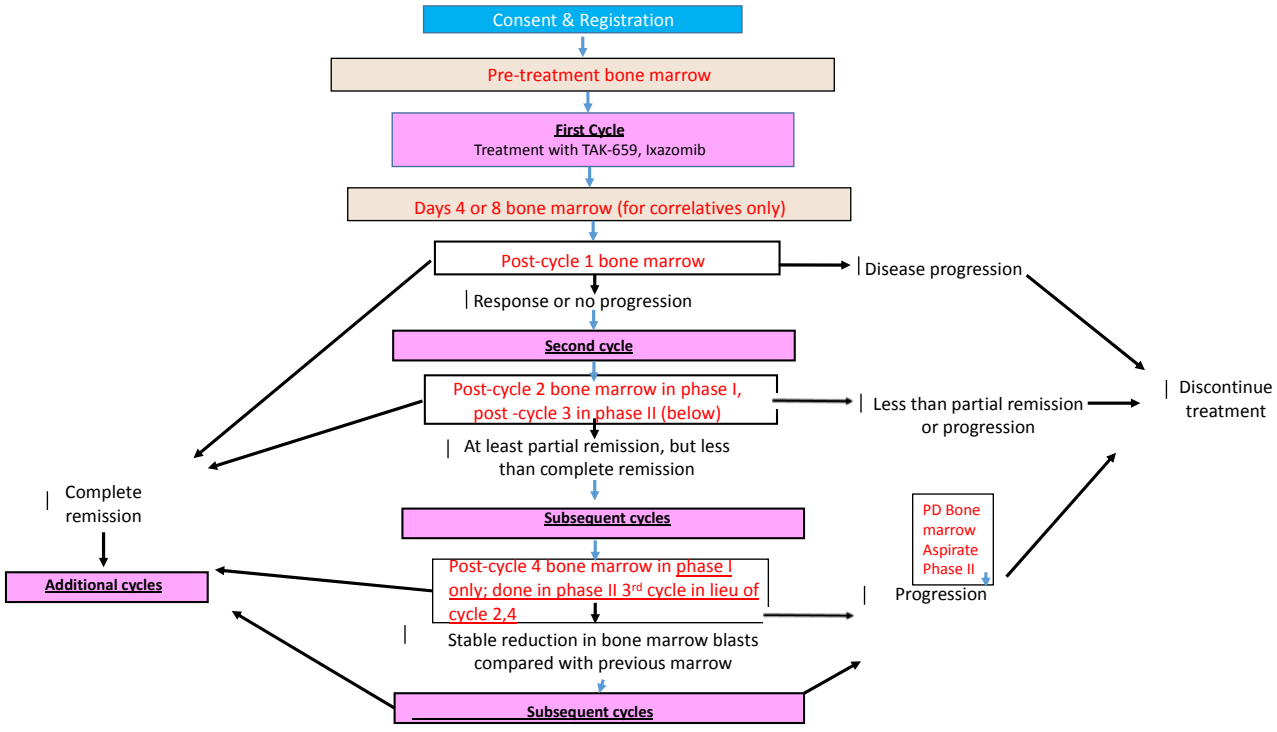


STUDY OVERVIEW DIAGRAMS

Phase II FLT-3- WT vs. -MUT cohorts:



SUBJECT SCHEMA FOR THERAPY



LIST OF ABBREVIATIONS AND GLOSSARY OF TERMS

Abbreviation	Term
5-HT_3	5-hydroxytryptamine 3 serotonin receptor
AE	adverse event
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AML	acute myelogenous leukemia
ANC	absolute neutrophil count
aPTT	activated partial thromboplastin time
ASCO	American Society of Clinical Oncology
ASCT	Allogenic stem-cell transplant
ASH	American Society of Hematology
AST	aspartate aminotransferase
AUC	area under the plasma concentration versus time curve
$AUC_{24\ hr}$	area under the plasma concentration versus time curve from zero to 24 hours
AUC_{inf}	area under the plasma concentration versus time curve from zero to infinity
AUC_{τ}	area under the plasma concentration versus time curve during the dosing interval
BCR	B-cell receptor
BCRP	breast cancer resistance protein
βhCG	beta-human chorionic gonadotropin
BID	bis in die; twice a day
BIW	Twice weekly
BSA	body surface area
BUN	blood urea nitrogen
BZD	Benzodiazepines
CBC	complete blood count
CFR	Code of Federal Regulations
CL	clearance, IV dosing
CLL	Chronic lymphocytic leukemia
CLM	Correlative Laboratory Manual
CL_R	Renal clearance
CL_P	plasma clearance
CL_{SS} /F	apparent clearance at steady-state after extravascular administration

Abbreviation	Term
CL_{Total}	total clearance
C_{max}	maximum (peak) observed concentration
CNS	central nervous system
CO_2	carbon dioxide
CPK	Creatinine phosphokinase
CR	complete remission ***or*** complete response
CRM	continual reassessment method
CRP	C-reactive protein
CSF-1R	colony-stimulating factor 1 receptor
CT	computed tomography
C_{trough}	end of dosing interval (trough) concentration
CV	cardiovascular
CYP	cytochrome P_{450}
DDI	Drug-drug interaction
DLBCL	Diffuse large B-cell lymphoma
DLT	dose-limiting toxicity
DNA	deoxyribonucleic acid
EBV	Epstein-Barr Virus
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	electronic case report form
EDC	electronic data capture
ELISA	enzyme-linked immunosorbent assay
EU	European Union
FDA	United States Food and Drug Administration
FLT3	FMS-like tyrosine kinase-3
GCP	Good Clinical Practice
G-CSF	granulocyte colony stimulating factor
GGT	gamma glutamyl transferase
GI	Gastrointestinal
GLP	Good Laboratory Practices
GM-CSF	granulocyte macrophage-colony stimulating factor
GMP	Good Manufacturing Practice
Hb	Hemoglobin
Hct	Hematocrit

Abbreviation	Term
HDPE	high-density polyethylene
hERG	human ether-à-go-go related gene
HIV	human immunodeficiency virus
HNSCC	Head and neck squamous cell carcinoma
IB	Investigator's Brochure
IC_{50}	concentration producing 50% inhibition
ICF	informed consent form
ICH	International Conference on Harmonisation
IEC	independent ethics committee
IRB	institutional review board
ITAM	immunoreceptor tyrosine-based activation-motifs
IV	intravenous; intravenously
IVRS	interactive voice response system
K_i	inhibition constant
KPS	Karnofsky Performance Status
LDH	lactate dehydrogenase
LFT	liver function test(s)
MAPK	Mitogen-activated protein kinase
MedDRA	Medical Dictionary for Regulatory Activities
MDSC	myeloid-derived suppressor cells
MRI	magnetic resonance imaging
MTD	maximum tolerated dose
MUGA	multiple gated acquisition (scan)
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NF-kB	nuclear factor kappa-B
NGS	Next Generation Sequencing
NK	Normal karyotype
NHL	non-Hodgkin lymphoma
NPO	nothing by mouth
NSCLC	non-small cell lung cancer
NYHA	New York Heart Association
PBMC	peripheral blood mononuclear cell

Abbreviation	Term
PCR	polymerase chain reaction
PD	Pharmacodynamics
Pgp	P-glycoprotein
PK	pharmacokinetic(s)
PO	*per os*; by mouth (orally)
PR	partial response
PRO	patient-reported outcome
PSA	prostate-specific antigen
pSYK	phosphorylated SYK
QD	*quaque die*; each day; once daily
QID	*quater in die;* 4 times a day
QOD	*quaque altera die*; every other day
QOL	quality of life
QTc	rate-corrected QT interval (millisec) of electrocardiograph
RBC	red blood cell
RECIST	response Evaluation Criteria in Solid Tumors
SAE	serious adverse event
SC	subcutaneous
SD	stable disease
SmPC	summary of Product Characteristics
SYK	spleen tyrosine kinase
$t_{1/2}$	terminal disposition half-life
TGI	tumor growth inhibition
T_{max}	time of first occurrence of Cmax concentration
TNBC	triple-negative breast cancer
ULN	upper limit of the normal range
US	United States
WBC	white blood cell
WHO	World Health Organization

1. BACKGROUND AND RATIONALE

1.1 Summary

This trial was conceptually founded on the results of a recently completed clinical trial from our institution addressing relapsed/refractory AML. That trial had involved use of a combination of targeting agents: acting at the levels of signal transduction (Flt3/Raf-selective inhibitor Sorafenib), and chromatin remodeling (Vorinostat and Bortezomib). Results from that trial demonstrated to us that, at least in Flt3ITD-positive disease, chemotherapy-free remissions could be achieved by cyclical administration of the combination, and the remissions were associated with a pretreatment gene expression signature of resistance effectors, whose expression-related impact was reversed in a very early phase of treatment initiation to predict success. In fact, introduction of Bortezomib was pivotal in enhancing the rate and depth of responses in our cases. We found early reversal of the resistance signature resulted in an inverted signature that characterizes good-risk core-binding factor AML, a form known to be curable with conventional chemotherapy when presenting de novo. The conclusion that the signature reversal we observed may be clinically meaningful in this relapsed/refractory stage was validated not only by rapidly occurring (within 1-2 weeks) deep morphologic eradication of bone marrow blasts, but by the occurrence of a pathway to successful transplant consolidation or to an unmaintained remission.

It appears likely that the concept for targeted AML therapy consisting of a combination of epigenetically-active agents that may precede not only transplant consolidation in relapsed/refractory AML, but prior to ("sensitivity priming") induction chemotherapy in de novo cases is germaine, because single targeted agents have not addressed the impact of combined mutational interaction involving signaling and chromatin-modeling effectors that is intrinsic to AML development. A combination of agents acting at the nexus of influence by mutations occurring in signaling and chromatin effectors that elicit resistance pathways, is needed so that resistance phenotype may be effectively reprogrammed.

Here, we have proposed a combination strategy using TAK-659 with Ixazomib because of extensive experience with agents, like Bortezomib, impacting pivotal targets whose influence is also neutralized by TAK-659 plus Ixazomib. This combination is likely to result in not only morphologic remissions, but also sensitivity priming for subsequent consolidation therapies delivered in near sequence. Indeed, most experts believe that continuous administration alone of single agent or even targeted therapy combinations, except in acute promyelocytic leukemia, is premature, and that the only rational application in high-risk AML is to plan cyclical administrations of 2-3 agents with a significant dose intensity. Given that TAK-659 and, particularly, Ixazomib have defined RP2D, and that they have only partial overlap in main toxicities, it is our strategy to perform a 3+3 escalation phase I trial beginning with the RP2D of TAK-659 in a shortened schedule compared to the current phase Ib duration, and to initiate Ixazomib with a one-dose step down from RP2D, in order to hasten the early identification of meaningful activity of the combination. This will allow for leukemic cytoreduction, and then a rest phase to allow regrowth of normal marrow precursors while avoiding toxicity from chronic exposure. Based on extensive experience with this type of approach from our prior trial, it is important to note that dose-interruption during the induction phase of therapy should not be tied to the occurrence of neutropenia or thrombocytopenia, but, rather, transfusion of platelets at the time of weekly or twice weekly outpatient visits should be undertaken, and prophylaxis against neutropenic infections is given, with a rule for admission for high-risk febrile episodes.

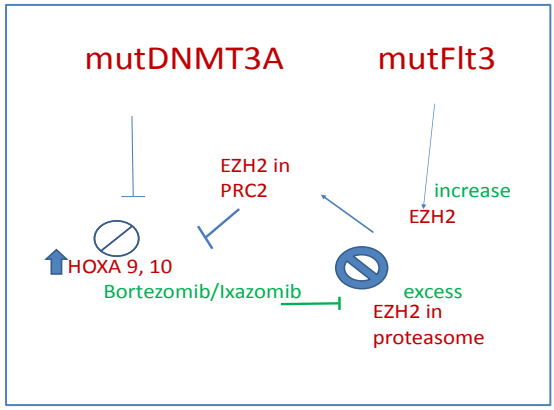
1.2 Acute Myeloid Leukemia: Current Standard of Care

Accumulating evidence points to initial clinical efficacy for single targeted agents in AML patients, when their primary blasts are annotated for single mutations as target. However, it has become clear that targeting must address at least two cooperative driver mutations in most AML cases, because single agents have only short-term benefit. Distinct patient outcomes have also been observed following uniform intensive chemotherapy for AML subsets described by pairing of driver mutations (1). However, the underlying origins for these chemotherapy outcomes are remote and reflect complex pathways, even when supplemented with single targeted agents as adjunct. Limited data exist to suggest an effective, and lasting combination targeted therapy in the context of paired driver mutations. Only then may targeting neutralize mutational impact on unsatisfactory chemotherapy outcomes. Investigation is needed to consider the leukemogenic program resulting from the summed burden of mutant drivers in the context of targeted therapy. Flt3-selective tyrosine kinase inhibitors (TKI's), as monotherapy, and along with chemotherapy, have perceived utility in AML, eg. Flt3ITD+ve (a class 1 mutation). However, co-existing mutant epigenetic landscaping genes (class 2 mutations) within AML founder clones affect resistance of leukemic stem cells (LSCs), thus preventing sustained remission.

1.3 Acute Myeloid Leukemia Mutations

Poor outcomes are frequent among certain acute myeloid leukemias (AMLs) characterized by small numbers of cooperating mutations (1). In particular, this group represents the largest fraction of AMLs in young adults. The cooperating mutations commonly involve epigenetic mechanisms amenable to targeted interventions. Such targeted interventions may, collectively in combinations, but only rarely when given alone, reverse resistance both to single tyrosine kinase inhibitors (TKIs) (eg. Those for Flt3ITD) and to a range of chemotherapies (2). A notable example is the ability of proteasome inhibitor (PI), Bortezomib, to silence HoxA expression, which is a major resistance factor for AML (2).

Figure 1-1 (below). EZH2/PRC2 upregulation by PI



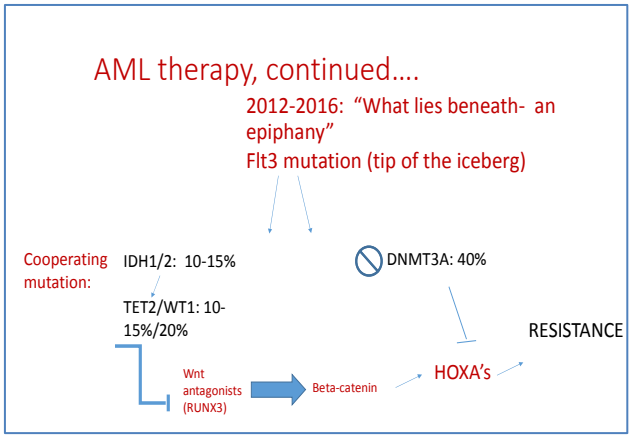
One of the earliest recognized, and most frequently occurring, driver mutations for AML is *Flt3ITD*. It does appear that prolonged maintenance administration of single TKIs may not be an optimal option for *Flt3ITD* AML as has proved to suffice for efficacy in chronic myeloid leukemia (CML).

Unlike the primacy of *BCR-ABL* mutation in the origins of CML, Flt3 tyrosine kinase oncogene mutations in AML arise late in the process of leukemogenesis and are most commonly preceded by mutations of epigenetic effectors such as *DNMT3A*, *ASXL1*, *TET2, IDH1/2, WT1* (3*)*. Mutations in these latter effectors, functioning at the level of chromatin and DNA, have a common end-function for promoting leukemic stem cell expansion, which involves master regulators from the *Hox-A* and *-B* alleles. Thus, a major attribute for *Flt3ITD* poor-risk AMLs *is HoxA/B* overexpression, and also expression of the TALE-factor collaborator *Meis1*, whose abundance distinguishes these AML subsets from those curable, but less frequent forms of AML with core-binding factor mutations (CBF+ve), which are largely devoid of *HoxA/B* and Meis1(4,5).

It is of crucial importance to emphasize the collaboration that occurs in <u>essentially all</u> cases of AML

bearing Flt3 mutations with one of the noted epigenetic modifiers (6).

Figure 1-2 (below). Virtually all Flt3 mutant AMLs have co-mutation of epigenetic effectors, affecting the Wnt pathway and HOXA locus

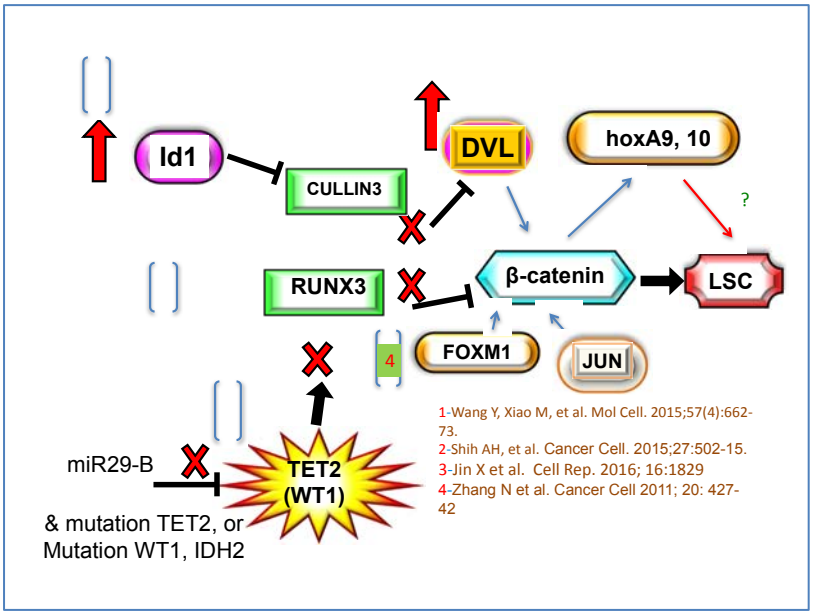


We have recently treated relapsed/refractory AML, in patients with a disease form bearing *Flt3ITD* and *TET2* mutations, by using a combination of the dual Flt3/raf inhibitor Sorafenib, the pan-histone deacetylase inhibitor Vorinostat, and the proteasome inhibitor Bortezomib. This combination, absent chemotherapy, proved to be quite effective at inducing severe depletion of blasts in the peripheral blood and marrow within a week's time, and this was preceded by early reversal of an intrinsic gene expression signature consisting of repressed *RUNX3*, and hyperexpressions of *Id1, c-jun, and HoxAs* (7).

Early gene signature inversion, in blasts from patients so treated, presaged remissions, which led to the possibility of curative allogeneic bone marrow transplant for patients achieving those remissions. All of these epigenetic effectors present in the signature link to the AML resistance phenotype marked by *HoxA* overexpression: 1) RUNX3 is a Wnt pathway inhibitor of β-catenin which is repressed by *TET2* mutation or other mutations in the group IDH1/2, WT1/TET2 (8,9); 2) Id1 is a Wnt pathway agonist of β-catenin downstream of Flt3 (via repression of Cullin E3 ubiquitin ligase)(10); and 3) c-jun and β-catenin can cooperate for *HoxA* transcription, and c-jun is known to transactivate *Meis1* (11-14) (Figure 1-3, below).

Therefore, we were led to wonder whether an even more potent combination might be devised to target these pathways for reversal of AML resistance, which may involve inhibition of a common pathway to allow re-establishment of that core-binding factor AML phenotype of therapeutic sensitivity marked by low/non-expression of *HoxAs/Meis1*.
Very recently, the non-receptor tyrosine kinase Syk was linked to the development of a resistance phenotype by *Flt3ITD+ve* AML, in part, via upstream and downstream effects at amplifying the Flt3-to-HoxA pathway (15). First, Syk is in a signaling complex, along with p85 PI-3 kinase, for Flt3ITD in AML (16).

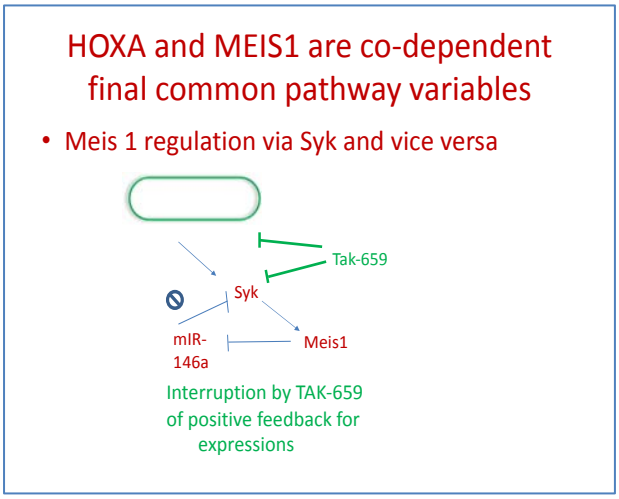
Figure 1-3.



Box1,2) RUNX3 is repressed in context of WT1/TET2 pathway inactivation, which prevents its antagonism of β-catenin nuclear function.

Box 3) Id1 is a repressor of Cullin, whose loss heightens DVL2, leading to β-catenin stabilization, which along with jun can bind and transactivate HOXA9/10.

Box 4) FOXM1, transactivated by jun (ENCODE) binds β-catenin and chaperones nuclear translocation (17) (additionally, jun and β-catenin heterodimerize for transactivation TCF4 motif) (14, *see below*).



In the receptor-related complex, mutual reciprocal cross-phosphorylations by Flt3 and Syk amplify the signal downstream to *c-myc*, among other targets. In addition, it was recently found that Meis1 upregulates Syk, via repression of miR-146a-a Syk modulator, and that Syk drives *Meis1* expression, resulting in a positive feedback loop (18). (**Figure** 1-4, left)

These features strongly recommend the addition of a dual Flt3/Syk inhibitor into a targeted agent combination trial for *Flt3ITD* AML. In addition, we and others have found the ability of dual Flt3/Syk inhibitors to bypass the TKD 835 mutation, which represents an advantage over Sorafenib used as the Flt3-selective TKI in our prior trial.

Flt3/Syk or Syk inhibitors also have demonstrated efficacy in disease subtypes of AML not involving *Flt3ITD.* For example, use of a dual Flt3/Syk inhibitor was able to cause in vitro differentiation of a variety of primary AML blast phenotypes (19). In addition, in an ongoing phase I trial utilizing a priming approach of administration of the Syk inhibitor entospletinib (GS-9973) as a lead-in prior to chemotherapy, it has been observed that one patient with a tMLL AML achieved complete remission prior to the onset of chemotherapy. (20) Such a result would seem to reflect a mechanism similar to that noted above in relation to downstream effects on HoxA/Meis1, which dominate the mechanism of tMLL AML.

Another crucial observation from our first trial was the dramatic impact Bortezomib made, when added to the therapeutic combination of Sorafenib with Vorinostat, upon the depth and rapidity of remissions

compared to the combination of Sorafenib and Vorinostat given in only a doublet combination (7). This has led us to speculate that the most important components of an epigenetically-active combination similar to the one we have previously studied, may in fact be the coupling of Bortezomib, or another proteasome inhibitor, with the TKI, which should optimally possess dual Flt3 and Syk selectivity.

Indeed, it is known that proteasome inhibitors degrade FOXM1 in several cancers and that FOXM1 is a necessary chaperone for β-catenin on Wnt/TCF4 target gene promoters, such as HOXA10 (17). This proteasome inhibitor activity against FOXM1 appears to occur as a result of upregulation of HSP70, which binds and sequesters FOXM1 protein, preventing it from positively autoregulating its promoter (21). The resulting inactivation of FOXM1 leads to loss of nuclear transport of β-catenin destined for Wnt/TCF4 promoters.

Further, we examined other putative measures for AML sensitivity to the combination used for the previous trial. In this regard, Sorafenib is not an active inhibitor of Syk kinase, and we found that Syk overexpression within blasts was a predictor for failure to achieve remission, where there was a 20-fold difference in transcript expression for Syk among responders vs. nonresponders (p=0.002). Several of these cases were found to be quite sensitive to a dual Flt3/Syk inhibitor when tested in vitro prior to commencing Sorefenib-based therapies in the patient (data not shown).

Our prior study using Bortezomib along with TKI is striking in its support for the central thesis of our proposed study. In addition, we have generated preliminary data to support the concept that TAK-659 is an attractive and efficacious agent for combination with proteasome inhibitors (Figs. 1-5 to1-9):

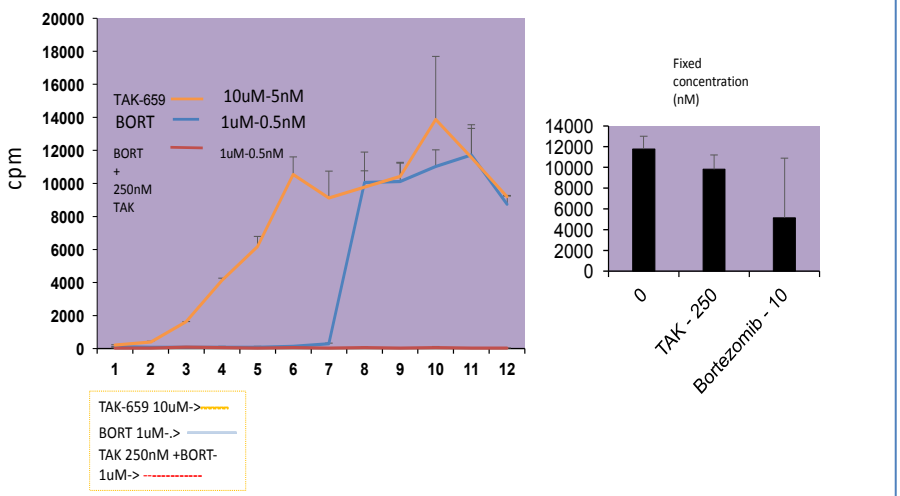


Figure : Synergy TAK-659 with Bortezomib in secondary AML: mJAK2V617F

In Figure 1-5, a patient treated on the Sor/Vor/Bor clinical trial failed to achieve a remission, but the marrow tested prior to onset of therapy was sensitive to TAK-659 and demonstrated enhanced cell extinction (visible apoptosis) measured by tritiated thymidine uptake with the combination of TAK-659 with Bortezomib over a range of concentrations.

Figure 1-5 (above). JAK2 mutant secondary AML is quite sensitive to TAK-659/Bortezomib.

In addition, we noted dramatic synergy of TAK-659 with Bortezomib in an aggressive de novo AML with the same functional phenotype as the Flt3ITD/TET2 mutation pair that we studied in our accompanying manuscript (WT1 mutation is the functional equivalent to TET2 mutation because they are binding partners and perform cooperative function [see ref (22)] (Figure 1-6, below).

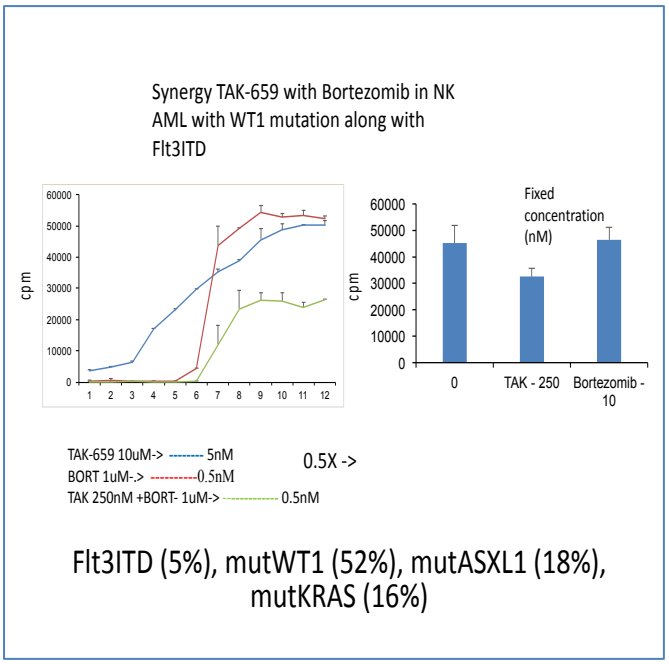


Figure1- 6 (right). Co-Occurrence of Flt3ITD with WT1/TET2 disruption sensitizes cells to TAK-659/Bortezomib combination in patient blasts #3220.

The same results were seen in another patient with high allele frequency WT1 mutation, again accompanying modest burden Flt3ITD mutation. This patient was found to be highly refractory to cycles of induction and reinduction chemotherapy, **including Midostaurin (Fig1-7, below)**.

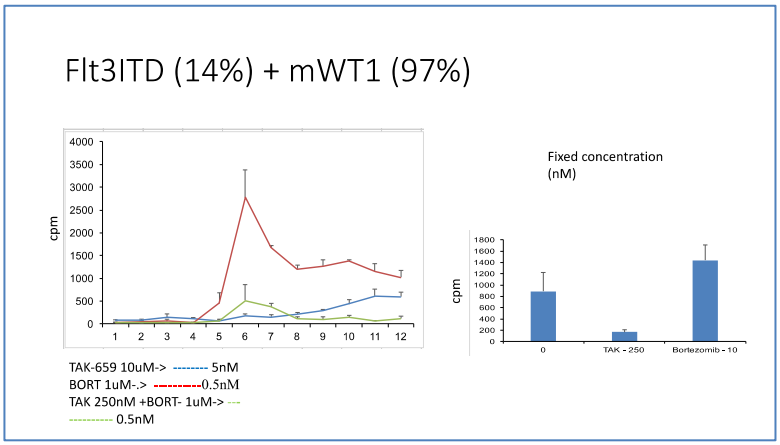
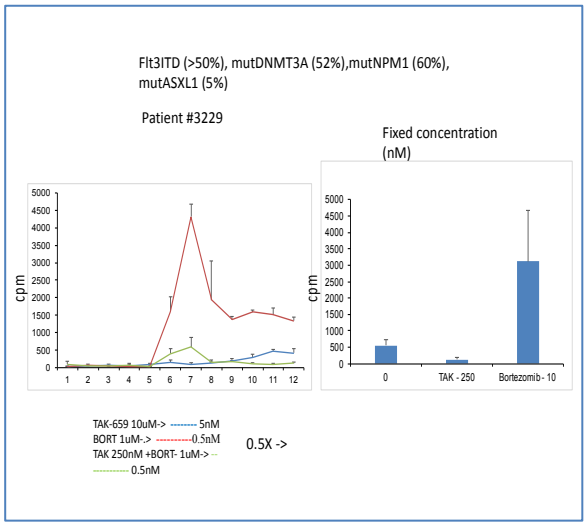


Fig. 1-7 (left). Low allele burden Flt3ITD plus WT1 mutation confers Midostaurin resistance, but sensitivity to TAK-659/Bortezomib at easily achieved plasma levels in patients.

Although Bortezomib alone had a propensity to dramatically inhibit proliferation and induce visible apoptosis of blasts when used alone at concentrations at or above 125 nM, at medium doses stimulation of cell survival occurred, which was overridden by the combination with TAK-659. At higher doses or very low doses of Bortezomib (the latter below 15nM) the combination of TAK-659 with Bortezomib was supradditive/synergistic for cell extinction (with visible apoptosis).

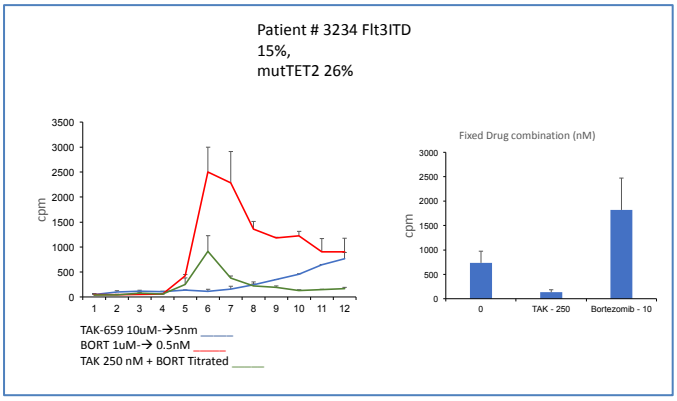
Figure 1-8. High allele burden Flt3ITD and DMT3A mutations sensitive to combination TAK-659/Bortezomib (below)



Further, based on the mechanism demonstrated for proteasome inhibition to silence HoxA9 via EZH2 in AML (2), we have also demonstrated strong synergism in vitro between TAK-659 and Bortezomib in a patient with high allele burdens of Flt3ITD and DNMT3A muations (Figure 1-8). In fact, DNMT3A mutation is a specific de-repressor for HoxA expression (1), occurring in 40% normal karyotype AMLs, which thus the combination TAK-659 and either Bortezomib or Ixazomib should target in those particularly risk-averse patients as demonstrated above. In fact, in this patient's blasts with extremely high burden of Flt3ITD (allele frequency >50% at diagnosis shown) and TAK-659 IC50 less than 19nM, the combination of Bortezomib with TAK-659 was better than TAK-659 save for 3 doses of Bortezomib in the combination.

Similarly, AML blast cells with the pairing of Flt3ITD with TET2 mutations, as previously reported, demonstrated the same synergy for antileukemic activity by TAK-659 and proteasome inhibitor.

Fig. 1-9 (right): Fl3ITD and TET2 mutant sensitivity to TAK-659/Bortezomib.



We have also demonstrated extreme sensitivity to TAK-659 in blasts of a patient with low allele burden of Flt3TKD mutation (2%) along with IDH2/DNMT3A mutations (50% allele burden each) with an IC50 TAK-659 of 150nM (data not shown).

<u>All these data validate conclusions we had made based on pretreatment *in vitro* testing of blasts from a patient (#14, Sor/Vor trial [7]) who was subsequently found to achieve a VGPR with Sor/Vor. That patient's blasts were tested in vitro with Sorafenib vs. R406 (Tamatinib, an investigational SYK inhibitor) in combination with either Vorinostat analogue SBHA or Botezomib. In that testing, tamatinib was superior in vitro to Sorafenib, and Bortezomib combination with tamatinib was vastly superior to the combination with SBHA.</u>

Thus, the underlying hypothesis derived from our experience is the following: Because of a known nuclear-to-cytoplasmic signal feedback loop that ties together Syk, HoxA, and Meis1, the combination of TAK-659 and proteasome inhibitors will target AMLs at this pivotal feedback junction where poor-risk AML is promoted:

a) upstream at the locus of Flt3/Syk receptor signal initiation, and

b) downstream at *HoxA/Meis1* transcription. These distinct inhibitors acting simultaneously are needed in order to achieve optimal responses in relapsed/refractory *Flt3ITD+ve* and Flt3ITD-*ve* AML.

In particular, Bortezomib and the similar proteasome inhibitor, Ixazomib, may act synergistically with TAK-659 to accomplish reversal of HoxA overexpression, and abolish *Meis1* transcription driven by c-jun and Syk, in the context of Wnt pathway activity that we have observed (7). The planned experiments pursue the detailed events relating the participants in this larger pathway to the superior outcome expected of the combination as compared to single agent impact on AML blasts with the drivers noted above. Particular attention will be paid to the impact of distinct mutant epigenetic modifiers (TET2, IDH1, IDH2, WT1, ASXL1,BCOR/BCORL1) which are known AML drivers of category 2, in their interaction with mutant drivers of category 1 acting within a TK pathway (Flt3mutant vs. in Flt3WT: KRAS, NRAS, JAK2, CBL).

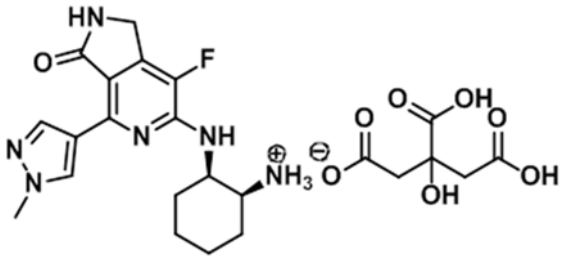
1.4 TAK-659

TAK-659 is an orally (PO) bioavailable, potent and reversible inhibitor of SYK and FLT3 currently under development for the treatment of patients with advanced malignancies.

SYK is a nonreceptor protein tyrosine kinase with SH2-binding domains that bind to phosphorylated immunoreceptor tyrosine activation motifs (ITAMs) located in B and T cells and certain natural killer (NK) cells. SYK becomes activated upon ITAM binding and subsequently controls the activity of downstream signaling pathways, eg, phosphoinositide 3-kinase (PI3K), mitogen-activated protein kinase (MAPK), and nuclear factor kappa-B (NF-kB) that mediate diverse cellular responses, including proliferation, differentiation, and survival. The SYK pathway is implicated in hematological tumors as well as select solid tumors development (eg, Epstein-Barr virus [EBV]-mediated naso-pharyngeal tumors).

FLT3 is a receptor tyrosine kinase that plays a role in hematopoiesis. Activating mutations in tyrosine kinase genes, including FLT3, are found in approximately 30% of patients with de novo acute myelogenous leukemia (AML). Mutations in the FLT3 gene most often involve internal tandem duplication of the juxtamembrane domain coding region or point mutations of the tyrosine kinase domain, resulting in ligand-independent proliferation due to constitutive activation of the FLT3 receptor conferring poor prognosis for patients (1, 7).

Figure 1-10. Chemical Structure of TAK-659



TAK-659 drug substance is a white to off-white crystalline powder. The pKa values were determined to be 0.95 (acidic) and 9.5 (basic).

1.4.1 Non-clinical Experience

1.4.1.1 Pharmacology (see Investigator's Brochure)

Nonclinical enzymatic and cell-based studies demonstrated that TAK-659 is a potent, reversible SYK and FLT3 inhibitor. TAK-659 inhibited SYK and FLT3 purified enzymes with a concentration producing 50% inhibition (IC_{50}) of 3.2 and 4.6 nM, respectively. Among a panel of kinases also

inhibited by TAK-659, the IC_{50} ranged from 1.4- to 115-fold higher than those for SYK. In addition, in a study of the cellular kinase selectivity of TAK-659, the IC_{50} for TAK-659 in a cell line expressing SYK was 116 nM, which was lower than the IC_{50} for any other kinase tested in this model (about 2-fold lower than the next lowest IC_{50} for ROS).

TAK-659 inhibited the B-cell receptor (BCR) signaling pathway, as indicated by inhibition of the autophosphorylation of SYK, as well as inhibition of the phosphorylation of several downstream targets. TAK-659 potently inhibited SYK and FLT3 activities in hematopoietic malignancy-derived, cultured human cell lines, including T-cell lymphoblastoma, megakaryoblastoma, and AML, with a concentration producing half-maximal response (EC_{50}) ranging from 11 to 775 nM in sensitive cell systems.

TAK-659 demonstrated significant antitumor activity after oral (PO) administration to xenografts: MV-4-11 (AML cell line model; 30 and 60 mg/kg; $p<0.001$), KG-1 (AML cell line model; 60 mg/kg; $p<0.001$), Additionally, pharmacodynamic analysis of MV-4-11 tumors from mice treated with TAK-659 demonstrated an apparent reduction of SYK phosphorylation at tyrosine residues Y525/526 (phosphorylated SYK [pSYK] Y525/526), as well as an increase in terminal biomarker cleaved caspase-3, CC3, a critical marker of apoptosis.

1.4.1.2 Pharmacokinetics

In nonclinical species, TAK-659 has good oral bioavailability, low plasma protein binding, moderate to large volumes of distribution, and moderate to high plasma clearance. TAK-659 was relatively stable with low extent of metabolism in human liver microsomes, S9, and hepatocytes; therefore, the metabolism is expected to be low in humans. TAK-659 is a major substrate of CYP3A4/5 and P-gp; thus, there is potential for an interaction between the corresponding inhibitor and inducer drugs. TAK-659 is not anticipated to perpetrate CYP- or P-gp/BCRP-mediated interactions in the clinic.

1.4.1.3 Toxicology

The nonclinical toxicology assessment of TAK-659 supports clinical trials in patients with advanced malignancies. GLP-compliant studies were conducted in accordance with ICH S9 guidance. In summary, the TAK-659 nonclinical toxicology profile supports its use in patients with advanced malignancies. However, there was evidence of genotoxicity in the Ames assay.

On the basis of in vitro and in vivo safety pharmacology studies, TAK-659 has low potential for cardiovascular (CV), respiratory, or central nervous system (CNS) effects in patients.

Detailed information regarding the nonclinical pharmacology and toxicology of TAK-659 can be found in the Investigator's Brochure (IB).

1.4.2 Clinical Experience

TAK-659 is being evaluated in a number of indications including NHL, AML, and advanced solid tumors (including triple-negative breast cancer[TNBC], non-small cell lung cancer [NSCLC], and head and neck squamous cell carcinoma [HNSCC]). An overview of ongoing studies is given in the table below.

Table 1. Overview of TAK-659 Clinical Studies

Protocol No.; Status	Study Objective(s)	Study Design and Population	Dose, Regimen, Route, Duration
C34001; closed to enrollment	Primary: safety, tolerability, MTD/RP2D. Secondary: PK, pharmacodynamics, preliminary efficacy.	Open-label, multicenter, phase 1, dose escalation study of TAK-659 in adult patients with advanced solid tumors and lymphoma malignancies.	Increasing oral doses of 60 mg, 80 mg, 100 mg, and 120 mg QD were assessed during dose escalation; a RP2D of 100 mg QD is being further explored in patients with lymphoma during dose expansion.
C34002; closed to enrollment	Primary: safety, tolerability, and MTD/RP2D in the phase 1b dose-finding portion and preliminary efficacy in the phase 2 dose expansion portion. Secondary: PK, pharmacodynamic, and differential efficacy depending on FLT3 mutation.	Open-label, multicenter, phase 1b/2, dose escalation study of TAK-659 in adult patients with relapsed or refractory AML.	Starting dose of 60 mg QD; additional doses of 100 mg, 120 mg, 140 mg, and 160 mg QD; 60 mg BID and 80 mg BID evaluated;
C34003; ongoing	Primary: determine MTD/RP2D of TAK-659 in combination with nivolumab and efficacy of the combination. Secondary: safety, tolerability, and efficacy of the combination; PK of TAK-659.	Open-label, multicenter, phase 1b, dose escalation and dose expansion study of TAK-659 in combination with nivolumab in adult patients with advanced solid tumors	Starting TAK-659 dose of 60 mg QD; dose has been escalated to 100 mg QD. Once determined, RP2D will be used in expansion cohorts. Nivolumab: 3 mg/kg IV dosing over 60 minutes every 2 weeks (Days 1 and 15 of each 28-day cycle). If the 240 mg fixed-dose cohort is evaluated and deemed safe and tolerable, the dosing regimen may switch to 240 mg on the basis of change in clinical practice and discussion between the investigator and sponsor. For patients participating in the 2-week monotherapy run-in with TAK-659, the first dose will be on Cycle 1 Day 15.
C34004, ongoing	Primary: efficacy Secondary: progression-free survival and efficacy	Open-label, multicenter, phase 2 study in adult patients with relapsed or refractory DLBCL, beginning with a lead-in dose exploration phase with 2 TAK-659 dose regimens	Cohort 1 = TAK-659 100 mg QD in 28-day treatment cycles. Cohort 2 = TAK-659 increasing dose (every 28 days) beginning with 60 mg QD, followed by 80 mg QD, up to 100 mg QD.

Protocol No.; Status	Study Objective(s)	Study Design and Population	Dose, Regimen, Route, Duration
C34005; ongoing	Primary: to determine MTD/RP2D when administered with each of the combination partners Secondary: PK of TAK-659 and preliminary efficacy with each of the combination partners	Open-label, multicenter, phase 1b, dose escalation study of TAK-659 in combination with 1 of 5 combination partners (bendamustine, bendamustine + rituximab, gemcitabine, lenalidomide, and ibrutinib) in adult patients with NHL after at least 1 prior line of therapy	Starting dose of 60 mg QD; dose will be escalated to 100 mg QD until MTD is reached. Combination partners include: • Bendamustine: 90 mg/m2 administered IV over 10 or 60 minutes (depending on which formulation is used) on Days 1 and 2 of a 21-day cycle, up to 8 cycles. • Bendamustine + rituximab: 90 mg/m2 bendamustine administered IV over 10 or 60 minutes (depending on which formulation is used) on Days 1 and 2 of a 21-day cycle, up to 8 cycles; and 375 mg/m2 rituximab administered IV per local guidelines and labeling on Day 1 of a 21-day cycle, up to 8 cycles. • Gemcitabine: 1000 mg/m2 IV infusion over 30 minutes on Days 1 and 8 of a 21-day cycle. • Lenalidomide: 25 mg PO QD for Days 1 to 21 of a 28-day cycle. • Ibrutinib: 560 mg PO QD of a 28-day cycle.
C34007; ongoing	Primary: to determine safety, tolerability, and MTD/RP2D and to characterize plasma and urine PK Secondary: to evaluate preliminary efficacy	Open-label, multicenter, 2-part, phase 1 study in East Asian adult patients, including a dose escalation in patients with NHL and an expansion in patients with DLBCL	Starting TAK-659 dose of 60 mg QD; dose will be escalated to 100 mg QD and will then follow 20 mg increments until MTD and/or RP2D is reached. Expansion phase will use RP2D.

Protocol No.; Status	Study Objective(s)	Study Design and Population	Dose, Regimen, Route, Duration
C34008; ongoing	Primary: determine the MTD and/or RP2D of TAK-659 and venetoclax, and to evaluate safety and tolerability Secondary: PK and preliminary efficacy of TAK-659 and venetoclax	Dose Escalation Phase: adult patients with advanced NHL of any histology. Patients will be refractory or relapsed after at least 1 prior line of therapy with no effective standard therapy available. Safety Dose Expansion Phase: MTD/RP2D + venetoclax will be explored in 2 dose safety expansion cohorts, advanced DLBCL and FL.	TAK-659: planned 60 or 100 mg PO, QD (or intermediate dose levels if appropriate), plus one of the following venetoclax regimens administered in 28-day cycles after Cycle 1 (35 days): • 50 mg QD increasing to 200 mg QD by Day 16. • 50 mg QD increasing to 400 mg QD by Day 17. • 50 mg QD increasing to 800 mg by Day 19. Expansion phase will use MTD/RP2D of TAK-659 and venetoclax combination
MLN1117-1003; completed	Primary safety: to determine DLT and MTD/RP2D for MLN1117 when administered with each of the combination partners. Primary efficacy: to evaluate the ORR as the primary efficacy measure of MLN1117 in combination with each of the combination partners in patients with gastric or gastroesophageal adenocarcinoma	An umbrella study to evaluate MLN1117 in combination with taxanes (docetaxel or paclitaxel) and other investigational anticancer agents (including TAK-659) for the treatment of patients with previously treated advanced and metastatic gastric and gastroesophageal adenocarcinoma	Details are included in the MLN1117 Investigator's Brochure.

AML=acute myeloid leukemia, BID=twice daily, DLBCL=diffuse large B-cell lymphoma, DLT=dose limiting toxicity, MTD=maximum tolerated dose, NHL=non-Hodgkin lymphoma, ORR=overall response rate, PK=pharmacokinetics, QD=once daily dosing, RP2D=recommended phase 2 dose.

1.4.2.1 Pharmacokinetics

Preliminary plasma pharmacokinetics (PK) results are available from lymphoma, solid tumor, and AML patients enrolled in Studies C34001 and C34002. In addition, preliminary urine PK results are available from lymphoma and solid tumor patients enrolled in the dose escalation cohorts of Study C34001. TAK-659 is characterized by fast absorption (overall median T_{max} [time of first occurrence of C_{max} (maximum observed concentration)] of 2 hours) in patients with hematologic and nonhematologic malignancies. Moderate variability is observed among dose-normalized steady-state AUC_{τ} (area under the plasma concentration-time curve during the dosing interval) values in lymphoma, solid tumor, and AML patients (coefficient of variation of 20.0%, 43.5%, and 34.8%, respectively). An approximately dose-proportional increase in steady state AUC_{τ} was observed over the 60 to 160 mg range in patients with AML. Mean accumulation ratios ranging from 1.90-fold to 2.54-fold and mean peak-to-trough ratios ranging from 4.34 to 5.09 were observed across the study populations after repeated QD dosing for 15 days. Based on data in lymphoma and solid tumor patients, renal clearance accounted for about 30% of TAK-659 apparent clearance, and therefore at least about 30% of TAK-659 systemic clearance. Active tubular secretion appeared to be the predominant component of renal clearance, based on comparison of unbound renal clearance to glomerular filtration rate. Geometric mean terminal disposition half-life of 34.4 hours was determined in a single dose PK run-in phase of the indolent NHL expansion cohort of Study C34001. Additional details on TAK-659 PK are provided in the TAK-659 IB.

A preliminary population PK model simulated results predicted 44% vs 30% of pts (N=2000) exposed to 80 mg BID vs 160 mg QD, respectively, would have a steady-state trough concentration of >100 ng/mL, a level leading to >90% pFLT-3 inhibition per (plasma inhibitory activity for Flt3mutant cell line in vitro) PIA(23, 24); thus forming the basis for evaluating the BID dose schedule.

1.4.3 Drug- Drug and Drug-Food Interactions

1.4.3.1 Drug-Drug Interactions

To date, no drug-drug interaction (DDI) studies have been conducted in humans. Because the metabolic and disposition pathways of TAK-659 remain to be fully characterized in humans in vivo, the risk of PK DDIs between TAK-659 and concomitantly administered drugs has been informed by data obtained from human in vitro systems.

In vitro studies indicate that TAK-659 is a substrate of P-gp and is metabolized by CYP3A4/5, CYP2D6, and CYP1A2, with relative contributions of 69.1% to 73.0%, 16.6% to 30.9%, and 0% to 8.40% for these respective CYPs. There is a potential risk for TAK-659 PK to be altered by drugs that are strong CYP3A inhibitors or inducers, or P-gp inhibitors or inducers. Consequently, until such potential interactions can be assessed in vivo, concomitant treatment with CYP3A strong inhibitors or inducers or with P-gp inhibitors or inducers should be avoided in clinical studies of TAK-659. Treatment with CYP3A strong inhibitors or inducers or P-gp inhibitors or inducers must be discontinued within a designated timeframe before the first dose of TAK-659 as specified in the individual study protocols.

In cell-based assays, TAK-659 was not a substrate of the efflux transporter BCRP or the uptake transporters OAT1, OAT3, OCT2, OATP1B1, or OATP1B3. Therefore, there is low predicted risk for inhibitors or inducers of these transporters to affect TAK-659 exposure.

1.4.3.2 Drug-Food Interactions

To date, the effect of food on the PK of TAK-659 has not been characterized in humans. Accordingly, TAK-659 should be administered on an empty stomach, at least 1 hour before and no sooner than 2 hours after food and beverages except water.

Because grapefruit juice is considered a strong CYP3A inhibitor, there is a potential risk for TAK-659 PK to be altered by grapefruit-containing food and beverages. Accordingly, consumption of grapefruit-containing food and beverages is prohibited during clinical studies of TAK-659.

1.4.4 Clinical Pharmacodynamics

Pharmacodynamic markers are being assessed across TAK-659 studies. In the C34002 AML study specifically, assessments of TAK-659 activity on FLT3 signaling pathway levels have been examined through plasma inhibitory activity assays. The results presented reflect data across multiple QD and BID dose levels as of October 2017 and the degree of FLT3 inhibition observed at the Cycle 1 Day 15 time point, the first time point at which FLT3 inhibition is measured after control.

The level of pFLT3 inhibition achieved at QD dose levels per plasma inhibitory activity assay was not consistently reaching 90%, a level of target modulation beyond which is believed to be necessary to achieve clinical efficacy in evaluation of single-agent Flt3 inhibitor administration (23). Therefore, an alternative BID dosing schedule was evaluated in the C34002 AML study, with a total daily dose not exceeding 160 mg.

The BID regimen proved advantageous over the QD regimen with respect to FLT3 inhibition, as 3 of the 4 patients at 80 mg BID achieved at least 90% inhibition of FLT3 by Cycle 1 Day 15. Whether this earlier and improved level of FLT3 inhibition translates to enhanced clinical activity remains to be seen. Analysis of additional cohorts (60 mg BID) and patient samples from this study was also pursued and is under analysis.

1.4.5 Clinical Efficacy

In Study C34002, 26 patients were evaluable for response, with 6 patients achieving >90% FLT3 inhibition at Cycle 1 Day 15 (100 mg QD, n = 1; 120 mg QD, n = 1; 160 mg QD, n = 1; 80 mg BID, n = 3). An additional 7 patients achieved >90% FLT3 inhibition at a subsequent time point. Early signs of clinical activity were observed in both FLT3-mutated and FLT3-WT patients. Three patients achieved response per International Working Group criteria at higher TAK-659 dose levels: 1 CR (FLT3-WT/160 mg QD) and 2 CRs with incomplete hematologic recovery (FLT3-WT/140 mg QD; FLT3-ITD/160 mg QD). An additional 6 patients achieved >50% bone marrow blast reduction from baseline without bone marrow recovery (n = 2, FLT3-ITD/100 mg QD; n = 1, FLT3- tyrosine kinase domain/120 mg QD; n = 1, FLT3- tyrosine kinase domain/160 mg QD; n = 2, FLT3-ITD/80 mg BID). The FLT3-WT patient who achieved CR at Cycle 4 maintained the CR through Cycle 10 despite achieving a maximal FLT3 inhibition of only 47% (Cycle 1 Day 15), suggesting that SYK inhibition may have contributed to this patient's response (24).

1.4.6 Summary of Safety

In Study C34001, the TAK-659 dose was escalated from 60 to 120 mg QD (60 mg [10 patients], 80 mg [4 patients], 100 mg [107 patients], and 120 mg [7 patients]). The MTD for patients with lymphoma and solid tumors is 100 mg QD. Expansion cohorts for patients with lymphoma were opened in December 2015, and patients in the expansion phase of the study are treated at the MTD/RP2D of 100 mg.

Of the 128 patients treated in Study C34001 (109 lymphoma [including 5 patients with CLL] and 19 solid tumors), 109 patients had discontinued from the study as of 22 October 2017. Reasons for discontinuation included progressive disease (PD) (51 patients), AEs (34 patients), symptomatic deterioration (14 patients), withdrawal by patient (2 patients), initiation of stem cell transplant (2 patients), protocol violation (1 patient), and other (5 patients). Nineteen patients were still receiving study drug as of the data cutoff date.

In Study C34002, the TAK-659 dose has been escalated from 60 to 160 mg (60 mg [4 patients], 100 mg [7 patients], 120 mg [4 patients], 140 mg [5 patients], 160 mg [9 patients], and 80 mg BID [6 patients]). The dosing C34002, starting at 60mg po qd, and expanding up to and including 80mg po bid and a subsequent cohort testing 60mg po bid, were an expansion phase I escalation intended to test not only tolerance, but also PK and a PD measure (Plasma inhibitory activity tested on a model cell line in vitro) as well as preliminary efficacy. Adverse events largely occurred at doses beginning at 160mg po qday and above. The doses of 60mg po and 100mg po daily were considered safe and the major reason for stopping included the occurrence of infections after several 28 day cycles. This, of course, is an inevitable outcome of continued myelosuppressive targeting agent therapy delivered for AML induction, which is problematic across the full spectrum of Flt3-selective tyrosine kinase inhibitor therapy without a rest period, including the experience of gilteritinib and quizartinib, which have been given in continuous dosing schedules.

In clinical trials, the incidence of grade =/ >3 pneumonia and sepsis were 35% with gilteritinib; or febrile neutropenia, pneumonia and sepsis of 48% with quizartinib. During study of both these agents the protocols had initially prohibited use of strong CYP inhibitors, which increase the drug levels of the TKIs. However, in the case of gilteritinib, the increased level affected by itraconazole as an example of strong CYP3A inhibitor was 120%. In both cases, avoidance was recommended but interruption of TKI therapy and/or significant dose reduction with stringent monitoring for toxicity in situations of necessity were allowed. An optimal strategy for mold-related infections has been use of isovuconazole, a moderate CY3A inhibitor.

Of the 35 patients treated in C34002 with continuous dosing schedule, 33 patients had discontinued from the study as of the data cutoff date. The reasons for discontinuation included AEs (21 patients), PD (5 patients), withdrawal by patient (2 patients), and other (5 patients). As regards DLTs, at the time of the last clinical reporting (K Pratz, et al Blood 2017 130:2622] [ref. 24[) (data cutoff 06Oct17), there were only two defined DLTs observed on study (35 patients)- one at 160mg QD (GR 3/4 amylase/lipase) and one at 80mg BID (Gr 3 bleeding).

Since that data cutoff, an evaluation of the QD and BID doses was performed at an End of Cohort Meeting in May 2018. At this meeting, per the 3+3 design, 160mg QD was considered the MTD and 140mg QD was considered the RP2D for QD dosing as determined by sponsor and investigators. 80mg BID was considered above the MTD having observed 2 DLTs at this dose level (Gr 3 bleeding, Gr 3 amylase/lipase), therefore 60mg BID was considered the MTD/RP2D for the BID single agent dosing regimen. A grade 3 pancreatitis event (with associated grade 3 lipase and amylase increase) occurred in one patient dosed at 100mg QD on day 1 of cycle 2 (day 29 overall) but this patient had comorbid Crohn's disease, acute kidney injury and concomitant use of amphotericin. These factors would be considered fully exclusionary for the continuation of study medication currently, as renal failure causes

significant accumulation of TAK-659 levels. Nevertheless, that investigator discontinued TAK-659 and the episode resolved, and the event was not considered directly related to study drug. Thus, there were no DLTs identified at the 100mg QD dose under acceptable rules for our study.

In Study C34003, the TAK-659 dose has been escalated from 60 to 100 mg (60 mg [6 patients], 80 mg [8 patients], and 100 mg [5 patients]). Of the 16 patients treated in this study, 11 patients had discontinued from the study as of the data cutoff date (Juric, D, unpublished data, 2017). In Study C34005, the TAK-659 dose has been escalated from 60 to 100 mg (TAK-659 + bendamustine [6 patients], TAK-659 + bendamustine/rituximab [6 patients], TAK-659 + gemcitabine [3 patients], TAK-659 + lenalidomide [3 patients], and TAK-659 + ibrutinib [1 patient]). Of the 19 patients treated in this study, 17 patients had discontinued from the study at the time of this report including 13 patients who discontinued due to PD (Assouline, S, unpublished data, 2017).

TEAEs, from a pooled analysis of the larger studies (C34001 and C34002), were generally as expected based on the patient population being studied and the nonclinical toxicology findings of TAK-659. In these studies, as of 22 October 2017, the most common TEAEs (reported in ≥30% of patients) were AST increased (92 patients [56%]), pyrexia (78 patients [48%]), amylase increased (64 patients [39%]), diarrhea (61 patients [37%]), anemia (57 patients [35%]), fatigue (55 patients [34%]), lipase increased (54 patients [33%]), hypophosphataemia (54 patients [33%]), and ALT increased (52 patients [32%]). The most common treatment-related TEAEs (reported in ≥20% of patients) were AST increased (76 patients [47%]), amylase increased (57 patients [35%]), lipase increased (50 patients [31%]), ALT increased (42 patients [26%]), blood CPK increased (38 patients [23%]), 31iarrhea (36 patients [22%]), and hypophosphataemia (36 patients [22%]).

The most common Grade 3 or greater TEAEs (≥10% of patients) have been amylase increased (35 patients [21%]), anemia (35 patients [21%]), hypophosphataemia (31 patients [19%]), neutropenia (29 patients [18%]), lipase increased (28 patients [17%]), febrile neutropenia (23 patients [14%]), thrombocytopenia (17 patients [10%]), AST increased (16 patients [10%]), and blood CPK increased (16 patients [10%]). The most common Grade 3 or greater treatmentrelated AEs (≥5% of patients) have been amylase increased (31 patients [19%]), lipase increased (25 patients [15%]), hypophosphataemia (24 patients [15%]), neutropenia (18 patients [11%]), blood CPK increased (15 patients [9%]), AST increased (10 patients [6%]), and anemia (9 patients [6%]). Further investigations are required to determine the clinical significance of the laboratory abnormalities, many of which have been asymptomatic, such as increased amylase, lipase, AST, ALT, and blood CPK.

As regards C34002 specifically, however, grade 3+ AST increases and ALT increases occurred in only 9% and 11%, respectively, across all dose levels 140qd-160qd-80bid-60 bid, and no DLTs were observed at 60mg and 100mg in this category.

Amylase and lipase grade 3 increases occurred in only 9 and 11%, respectively, and were absent, except as noted above, from 60mg and 100mg qd cohorts. Gastric hemorrhage occurred at 80mg bid in one case as noted. In addition, 13/35 (37%) patients received equal to or greater than 3x 28 day cycles of TAK-659 at the stated dose.

Two cases of pancreatitis were reported (both in Study C34002). One case, as noted above, was reported as a Grade 3 serious adverse event (SAE) occurring 29 days after first dose of TAK-659 and considered

by the investigator to be possibly related to study treatment, albeit with several confounding factors (eg, AML, Crohn's disease, acute kidney injury, and concomitant use of amphotericin B). The patient had experienced abdominal discomfort but denied severe abdominal pain, nausea, vomiting, diarrhea, or other GI problems. An imaging workup also showed peripancreatic edema. The event resolved within 16 days of diagnosis, and the patient resumed TAK-659 at a reduced dose. The patient was discontinued from the study within the following month for Grade 3 treatment-related rash. The second case of pancreatitis was a Grade 2 AE with only laboratory enzyme elevation and unrelated Grade 1 abdominal distension. This patient subsequently died due to progression of AML. Changes in pancreatic enzymes continue to be monitored with high frequency in Cycle 1 and then at least once per month across studies.

LDH increases have been observed in almost all patients across ongoing studies. There has been no obvious correlation with dose level, tumor type, baseline LDH levels, or relationship to PD. No dose modifications have occurred strictly based on LDH elevations; however, LDH values have generally decreased with a TAK-659 dose hold due to other reasons.

According to all available data sources, there have been 59 deaths: 39 patients in Study C34001, 17 patients in Study C34002, 2 patients in Study C34003 (Juric, D, unpublished data, 2017), and 1 patient in Study C34005 (Assouline, S, unpublished data, 2017). Six of the AEs that led to death were considered treatment related and are described in Section 5.5.7 of the current IB.

1.4.7 Summary of Toxicities and Potential Risks

1.4.7.1 Potential Risks Based on TAK-659 Clinical Studies

Lipase Elevations

Asymptomatic elevation in lipase is a potential risk of TAK-659. In nonclinical studies, lipase was sporadically elevated at high doses of TAK-659 in both rats and dogs; however, there was no evidence of microscopic organ damage. In Studies C34001 and C34002, 54 patients (33%) overall have reported a TEAE of lipase increased, and of those, 50 patients (31%) have reported treatment-related TEAEs of lipase increased. These included 1 Grade 4 and 1 Grade 3 lipase increased. The Grade 4 lipase increased had a corresponding event of amylase increased (Grade 3 laboratory result) and a concurrent event of unrelated Grade 1 cholelithiasis; the Grade 3 lipase increase did not have a corresponding event of amylase increased. A careful review of both cases revealed no evidence of pancreatitis or organ damage. In addition, lipase values declined with discontinuation of TAK-659. The lipase events were classified as DLTs, and per the protocol, the 60 mg cohort was expanded. The current protocols include frequent screening of serum lipase, and these values will continue to be closely monitored. As of 22 October 2017, 3 additional lipase events have been reported (Studies C34001 and C34002): 1 patient in the C34001 100 mg cohort (Grade 3 event), and 2 patients in Study C34002 (in the 160 and 80 mg bid cohort, respectively). As noted, there were no DLTs identified at the 100mg QD dose under acceptable guidance rules for our proposed study. In Study C34005, 16% of patients experienced an elevation (related or nonrelated, any grade) in lipase (Assouline, S, unpublished data, 2017). Asymptomatic elevation in lipase is included as a potential risk of TAK-659.

Pneumonitis

There were 5 SAEs of pneumonitis (4 reported in Study C34001 and 1 reported in C34002), which were all considered by the investigator to be possibly related to study drug. The first patient developed pneumonitis 46 days after starting treatment with TAK-659 at 80 mg QD. The event of pneumonitis was

considered possibly related to TAK-659 treatment; it was determined to be an unanticipated problem and was reported to health authorities. Pneumonitis was added as a potential risk of TAK-659, safety letters were sent to the study sites, and the drug safety information in the informed consent forms was updated. The event was the first SAE of pneumonitis in TAK-659 program. Subsequently, 2 SAEs of pneumonitis were reported during the previous reporting period: 1 patient with DLBCL who experienced pneumonitis 34 days after starting and 10 days after discontinuing treatment with TAK-659 at a dose of 100 mg QD, and another patient with CLL who experienced pneumonitis 3 months after starting TAK-659 at a dose of 100 mg QD. In the current reporting period, 2 new SAE cases of pneumonitis were reported (C34001 100 mg QD and C34002 160 mg QD). No cases of pneumonitis have been observed at 100mg QD in C34002. Pneumonitis has been reported in clinical experiences of other FLT3 and BCR pathway kinase inhibitors. No other safety signals or changes to the known benefit-risk profile of TAK-659 in relation to the additional cases of pneumonitis were identified from review of these SAEs. Investigators are advised to closely monitor patients for respiratory signs and symptoms throughout TAK-659 treatment.

Intestinal, Renal, and Lens Epithelial Effects and Related Epithelial Effects
In rats, the primary epithelial effects were lens epithelium disorganization/hyperplasia and renal and urinary bladder transitional epithelium hyperplasia. Correlating with changes in the urinary tract were decreases in urine volume. Throughout the digestive tract (from duodenum to rectum) and usually occurring in the crypts or at the base of the mucosa, there was single-cell necrosis with hemorrhages at nontolerated doses, Other effects on epithelial tissues included stomach glandular mucosa hemorrhage, lacrimal gland atrophy/necrosis, and mammary gland and skin hair bulb single-cell atrophy/necrosis. The lens hyperplasia had some single-cell necrosis and correlated with slight axial subcapsular anterior cortical lens opacity. All the epithelial effects in rats were reversing or reversed by the end of the 28-day recovery period, except for lens opacity.

In dogs, the primary epithelial effect was on the intestine and included hemorrhage of the intestinal mucosa (ileum, cecum, and/or colon). A dose-dependent increased incidence of liquid/soft feces was observed and was consistent with intestinal changes. All intestinal effects were reversed by the end of the 28-day recovery period.

During the clinical studies, patients will receive eye exams to monitor for any changes to the eye lens; effects on other epithelial tissues will be monitored via routine laboratory tests, urinalysis, physical examinations, and AE monitoring as detailed in the protocols.

Genotoxicity
TAK-659 was mutagenic in an Ames assay (*S typhimurium* TA1537) after metabolic activation with rat liver enzymes and was aneugenic in micronucleus evaluations. Increased micronucleus formation in human peripheral blood lymphocytes was primarily associated with positive kinetochore staining and, thus, was positive for aneugenic genotoxicity. SYK has been implicated in mitotic spindle function (25); therefore, the aneugenic effect of TAK-659 was consistent with its pharmacologic mechanism of action. Based on the genotoxic nature of TAK-659, its toxicity profile would be acceptable for patients with advanced malignancies.

1.5 Ixazomib (MLN9708)

1.5.1 Preclinical Experience

Please refer to the current ixazomib Investigator's Brochure (IB).

1.5.2 Clinical Experience

Ixazomib has been evaluated as an oral single agent in phase 1 studies that have included patients with advanced solid tumors, lymphoma, relapse/refractory MM (RRMM), and relapsed or refractory light-chain (AL) amyloidosis, as well as AML, and demonstrated early signs of activity. Ongoing studies continue to investigate both single-agent ixazomib and ixazomib in combination with standard treatments. Based on encouraging preliminary data observed in patients with MM requiring systemic treatment, 2 phase 3 trials in newly diagnosed MM (NDMM) (C16014) and RRMM (C16010) patient populations have evaluated ixazomib in combination with Revlimid and Dexamethasone (RevDex) versus placebo/RevDex. Both trials combine ixazomib at a weekly dose of 4.0 mg on Days 1, 8, and 15 in a 28-day cycle to a standard dose of lenalidomide with a weekly dexamethasone dose of 40 mg. In addition, clinical pharmacology studies have evaluated drug-drug interactions with ketoconazole, clarithromycin, and rifampin, as well as the effect of food, renal impairment, and hepatic impairment on the PK of ixazomib.

Fatigue was the most common AE reported among 384 patients treated in the oral (PO) studies (47%). Other common AEs reported in the pooled intravenous (IV) and PO safety populations include nausea, thrombocytopenia, diarrhea, and vomiting. Rash is also a commonly reported treatment-emergent event; however, there is some variety in its characterization and causality resulting in different preferred terms to describe it. A high-level term outline of rash events includes rashes, eruptions and exanthems NEC; pruritus NEC; erythemas; papulosquamous conditions; and exfoliative conditions. The dose escalation phases of most trials reported in the IB have now completed enrollment, and gastrointestinal (GI) symptoms were the common dose-limiting toxicities (DLTs) when the use of prophylactic anti-emetics was not permitted per protocol. In the expansion cohorts or phase 2 cohorts (as per each study), the incidence and severity of GI symptoms was mitigated by the use of the lower maximum tolerated dose (MTD)/recommended phase 2 dose (RP2D) (as per each study) and standard clinical usage of anti-emetics and/or antidiarrheal medications as deemed appropriate. Prophylactic use of anti-emetics has not been required as with other agents but (as outlined in Section 6.3) has been used according to standard practice and are effective.

In addition to the experience gained in myeloma, early data are available regarding attempts to blend ixazomib into chemotherapeutic salvage chemotherapy for AML (26, 27). However, the early phase I design of the initial trial lent only suggestive information regarding efficacy or impact on potential targets. A recent phase I trial of Ixazomib along with standard cytarabine/anthracycline (daunorubicin 60mg/m2) "7+3" ([27] PC Amrein et al. Blood ASH abstract 4059A, 2018) used a 3+3 design of cohorts at Ixazomib doses of 1-,2-,3-mg given twice weekly, and reached a dose of 3mg given on days 2,5,9, 12, with only 1 DLT (hematologic) in the 3mg cohort of 6 patients. Almost all AEs were hematologic, but 1 patient each in this treatment cohort had grade 3 elevation of ALT or AST, respectively. These data do not significantly differ from toxicities expected from the chemotherapy alone. It should be noted that in both trials above, twice weekly Ixazomib dosing was used, and that such twice weekly Ixazomib dosing schedules lead to significant accumulation based on the long half-life. By contrast, in single-agent phase I studies the MTD of the weekly dose of Ixazomib given alone on days 1,8,15 of a 28 day cycle was 2.97mg/m2 (ie. almost 6mg per dose). That is the schedule employed in this combination study, where the maximal dose would be the FDA-approved dose, which is roughly one-half that MTD, and the starting dose is two-step reduced. In addition, we have previously published the easy tolerability of Bortezomib at the full FDA-approved dose for myeloma, of 1.3mg/m2 IV day

1,4,8,11 (7), which is in accordance with the conclusion that accumulation pharmokinetics of Ixazomib, as compared to Bortezomib might explain any problems with twice weekly dosing in a combination setting.. No adverse events in the dose escalation phase of twice weekly administered Ixazomib with MEC chemotherapy were attributed to ixazomib alone (26). There is no evidence to suggest, based on our combination of Bortezomib using its maximal FDA-approved dosing combined with TKIs, that equal or greater than grade 3 toxicity might occur with the combination of Ixazomib added to TAK-659. No data are available on the cardiac toxicity of the combination of Ixazomib and TAK-659, but data on the QTc effect of Ixazomib single agent is summarized in reference (28).

Additional detailed information regarding the clinical experience of ixazomib may be found in the current IB.

1.5.3 Pharmacokinetics and Drug Metabolism

Source: Ixazomib Investigator's Brochure 11. (*see also, ref. 29, 30)*

After oral dosing, absorption of ixazomib is rapid with a median first time to maximum observed plasma concentration (T_{max}) of approximately 1 hour postdose. The plasma exposure (AUC) of ixazomib increases in a dose-proportional manner over a dose range of 0.2 to 10.6 mg based on population PK analysis. The absolute oral bioavailability (F) of ixazomib is estimated to be 58% based on population PK analysis. A high-fat meal reduced ixazomib C_{max} by 69% and AUC_{0-216} by 28%. This indicates that a high-fat meal decreases both the rate and extent of absorption of ixazomib. Therefore, ixazomib should be dosed at least 2 hours after food or 1 hour before food.

The steady-state volume of distribution of ixazomib is large and is estimated to be 543 L based on a population PK model. Based on in vitro plasma protein binding measurements on samples from clinical studies (Studies C16015 and C16018), ixazomib is highly bound to plasma proteins (99%). Ixazomib concentrations are higher in whole blood than in plasma, indicating extensive partitioning of ixazomib into red blood cells, which are known to contain high concentrations of the 20S proteasome.

Metabolism appears to be the major route of elimination for ixazomib. In vitro studies indicate that ixazomib is metabolized by multiple cytochrome P450 (CYP) and non-CYP proteins. At concentrations exceeding those observed clinically (10 μM), ixazomib was metabolized by multiple CYP isoforms with estimated relative contributions of 3A4 (42.3%), 1A2 (26.1%), 2B6 (16.0%), 2C8 (6.0%), 2D6 (4.8%), 2C19 (4.8%), and 2C9 (≤1%). At 0.1 and 0.5 μM substrate concentrations, which are closer to clinical concentrations of ixazomib following oral administration of 4 mg ixazomib, non-CYP mediated clearance was observed and seemed to play a major role in ixazomib clearance in vitro. These data indicate that at clinically relevant concentrations of ixazomib, non-CYP proteins contribute to the clearance of ixazomib and no specific CYP isozyme predominantly contributes to the clearance of ixazomib. Therefore, at clinically relevant concentrations of ixazomib, minimal CYP-mediated DDIs with a selective CYP inhibitor would be expected.

Ixazomib is neither a time-dependent inhibitor nor a reversible inhibitor of CYPs 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, or 3A4/5. Ixazomib did not induce CYPs 1A2, 2B6, and 3A4/5 activity or corresponding immunoreactive protein levels. Thus, the potential for ixazomib to produce DDIs via CYP isozyme induction or inhibition is low.

Ixazomib is not a substrate of BCRP, MRP2 and OATPs. Ixazomib is not an inhibitor of P-gp, BCRP, MRP2, OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1 and MATE2-K. Ixazomib is unlikely to cause or be susceptible to clinical DDIs with substrates or inhibitors of clinically relevant drug transporters.

The geometric mean terminal half-life ($t_{1/2}$) of ixazomib is 9.5 days based on population PK analysis. For both IV and oral dosing, there is an approximately average 3-fold accumulation (based on AUC) following the Day 11 dose for the twice-weekly schedule and a 2-fold accumulation (based on AUC) following the Day 15 dose for the once-weekly schedule.

Mean plasma clearance (CL) of ixazomib is 1.86 L/hr based on the results of a population PK analysis. Taken together with the blood-to-plasma AUC ratio of approximately 10, it can be inferred that ixazomib is a low clearance drug. Using the absolute oral bioavailability (F) estimate of 58% (also from a population PK model), this translates to an apparent oral plasma clearance (CL/F) of 3.21 L/hr. The geometric mean renal clearance for ixazomib is 0.119 L/hr, which is 3.7% of CL/F and 6.4% of CL estimated in a population PK analysis. Therefore, renal clearance does not meaningfully contribute to ixazomib clearance in humans. Approximately 62% of the administered radioactivity in the ADME study (Study C16016) was recovered in the urine and 22% of the total radioactivity was recovered in the feces after oral administration. Only 3.2% of the administered ixazomib dose was recovered in the urine as unchanged ixazomib up to 168 hours after oral dosing, suggesting that most of the total radioactivity in urine was attributable to metabolites.

The PK of ixazomib was similar with and without co-administration of clarithromycin, a strong CYP3A inhibitor, and hence no dose adjustment is necessary when ixazomib is administered with strong CYP3A inhibitors. Consistently, in a population PK analysis, co-administration of strong CYP1A2 inhibitors did not affect ixazomib clearance. Therefore, no dose adjustment is required for patients receiving strong inhibitors of CYP1A2. Based on information from the clinical rifampin drug-drug interaction (DDI) study, ixazomib C_{max} and $AUC_{0\text{-last}}$ were reduced in the presence of rifampin by approximately 54% and 74%, respectively. Therefore, the co-administration of strong CYP3A inducers with ixazomib is not recommended.

Mild or moderate renal impairment (CrCL ≥ 30 mL/min) did not alter the PK of ixazomib based on the results from a population PK analysis. As a result, no dose adjustment is required for patients with mild or moderate renal impairment. In a dedicated renal impairment study (C16015), unbound $AUC_{0\text{-last}}$ was 38% higher in patients with severe renal impairment or ESRD patients requiring dialysis as compared to patients with normal renal function. Accordingly, a reduced starting dose of ixazomib is appropriate in patients with severe renal impairment or ESRD requiring dialysis. Pre- and post-dialyzer concentrations of ixazomib measured during the hemodialysis session were similar, suggesting that ixazomib is not readily dialyzable, consistent with its high plasma protein binding (99%).

The PK of ixazomib is similar in patients with normal hepatic function and in patients with mild hepatic impairment, as defined by the National Cancer Institute Organ Dysfunction Working Group (total bilirubin <1.5 times the upper limit of normal [ULN]), based on the results from a population PK analysis. Consequently, no dose adjustment is required for patients with mild hepatic impairment. In a dedicated PK study in patients with moderate (total bilirubin >1.5 to 3 times the ULN) or severe (total bilirubin >3 times the ULN) hepatic impairment (Study C16018), unbound dose-normalized $AUC_{0\text{-last}}$

was 27% higher in patients with moderate or severe hepatic impairment as compared to patients with normal hepatic function. Therefore, a reduced starting dose of ixazomib is appropriate in patients with moderate or severe hepatic impairment.

There was no statistically significant effect of age (23-91 years), sex, body surface area (1.2-2.7 m^2), or race on the clearance of ixazomib based on the results from a population PK analysis.

Based on a pooled analysis of data from 4 phase 1 studies, ixazomib does not prolong the QTc interval or have a clinically meaningful effect on heart rate at clinically relevant exposures. (27) At the 4 mg dose, the mean model-predicted change from baseline in QTcF was 0.0710 msec (90% CI; -0.221, 0.363). Also, there was no discernible relationship between concentration and the RR interval, supporting the lack of a clinically meaningful effect of ixazomib on heart rate. These results collectively support the conclusion of low pro-arrhythmic risk associated with ixazomib.

Further details on these studies are provided in the IB.

1.5.4 Clinical Trial Experience Using the Oral Formulation of Ixazomib

As detailed in IB-11, preliminary clinical data are available as of 27 March 2017 for a total of 3623 patients across 26 studies; in these studies, 24 were studies with oral ixazomib. (The overall safety population comprises 941 patients who received at least 1 dose ixazomib [oral or IV formulation] in open-label studies, but aggregate data for SAEs and deaths are provided for an additional 2682 patients enrolled in the ongoing phase 3 Studies C16010, C16010CCS, C16011, C16014, C16014KES, C16019, and C16021. Therefore, for SAE and death data, the total number of patients is 3623.) The emerging safety profile indicates that ixazomib administration can lead to AEs that are generally manageable and reversible with dose reduction and supportive care. Additionally, the AEs in the combination studies are consistent with the safety profile of the individual agents in the combination regimen (eg, myelosuppression is common in regimens containing melphalan, and rash is common in regimens containing lenalidomide). While some of these potential toxicities may be severe, they can be managed by clinical monitoring and standard medical intervention. The AEs presented in this section are pooled from all dose regimens and dose levels. Results from clinical pharmacology studies support the continued exclusion of strong CYP3A inducers in the ongoing clinical studies [31] and that ixazomib should be administered on an empty stomach (at least 1 hour before or at least 2 hours after food). [32] Based on clinical experience from the phase 1 studies, the dose selected for the phase 3 studies is 4 mg given weekly for 3 weeks in a 28-day cycle.

Late-stage ixazomib development is currently focused on the oral formulation.

Rash, although a common AE in aggregate, was characterized in many ways; therefore, it is less common when considering individual preferred terms. Rash may range from limited erythematous areas, macular and/or small papular bumps that may or may not be pruritic over a few areas of the body, to a more generalized eruption that is predominately on the trunk or extremities. The frequency of rash was higher with the twice-weekly schedule and when ixazomib was combined with an agent where rash is an overlapping toxicity, such as lenalidomide. Data from Global Study C16010 demonstrate an incidence of 36% in the ixazomib+LenDex regimen and 23% in the placebo+LenDex regimen, with the difference between groups driven primarily by Grade 1 and 2 events. The difference in frequency of TEAEs in Skin and subcutaneous tissue disorders was primarily driven by the HLT Rashes, eruptions, and exanthemas not elsewhere classified (NEC) (20% and 13%, respectively). The rash events occurred

primarily in the first 3 months after initiation of the study regimen and were frequently self-limiting (21% of patients in the ixazomib+LenDex regimen and 12% of patients in the placebo+LenDex regimen reported the events resolved without intervention). [33] The first incidence of rash events occurred early during treatment, and there was no evidence of increased frequency of rash with prolonged exposure. The rare risks of Stevens-Johnson syndrome, toxic epidermal necrolysis (TEN), drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome, and pemphigus vulgaris have been reported in ixazomib-containing oncology studies (or blinded studies containing ixazomib or placebo) when given in a multi-therapy regimen in combination with agents associated with skin reactions (eg, Bactrim, aspirin, lenalidomide).

In Global Study C16010, antiviral agents for herpes zoster prophylaxis were allowed at the physician's discretion and were prescribed for 60% (431/720) of patients. The incidence of herpes zoster reactivation was 8% (11 patients) in the ixazomib+LenDex regimen and 3% (5 patients) in the placebo+LenDex regimen for patients who did not receive antiviral prophylaxis compared with <1% (2 patients) in the ixazomib+LenDex regimen and 1% (3 patients) in the placebo+LenDex regimen for patients who received antiviral prophylaxis. In the blinded C16019 study of ixazomib maintenance vs placebo in patients who have undergone ASCT (a population at high risk for herpes virus reactivation), there was an 8% incidence of herpes zoster, primarily in patients not receiving antiviral prophylaxis. In Study C16010CCS, less antiviral prophylaxis was used; herpes zoster was reported in 21% compared with 3% of patients treated with the ixazomib regimen and placebo regimen, respectively; all were reported as related to study treatment. Investigators should consider the use of antiviral prophylaxis for patient being treated with ixazomib.

1.5.5 Overview of the Oral Formulation of Ixazomib

The emerging safety profile indicates that ixazomib is generally well tolerated. The adverse events (AEs) are consistent with the class-based effects of proteasome inhibition and are similar to what has been previously reported with VELCADE though the severity of some, for example peripheral neuropathy, is less. While some of these potential toxicities may be severe, they can be managed by clinical monitoring and standard medical intervention, or, as needed, dose modification or discontinuation.

In the 4 ongoing studies (C16003, C16004, C16007, and C16009) investigating single-agent oral ixazomib in patients with differing malignancies (multiple myeloma, AL amyloidosis, nonhematologic cancers, and lymphoma), a total of 201 patients have been treated as of 27 March 2013.

These data for single agent, although of older study are the basis for unfettered interpretation for subsequent combination trials such as X34004 herein. These patients have been treated with different doses of ixazomib as they are all phase 1 trials.

The most frequent TEAEs (those reported in at least 10% of the total safety population, excluding phase 3 Studies C16011, C16014, C16014KES, C16019, and C16021), regardless of study drug causality, are described in the current ixazomib IB. The emerging safety profile indicates that ixazomib administration can lead to AEs that are generally manageable and reversible with dose reduction and supportive care. The most common AEs pooled across oral formulation studies (single agent and in combination) include nausea, diarrhea, fatigue, vomiting, thrombocytopenia, and constipation. Rash, although a common AE in aggregate, is characterized in many ways; therefore, it is less common when considering individual preferred terms. The rash may range from limited erythematous areas, macular and/or small papular

bumps that may or may not be pruritic over a few areas of the body, to a more generalized eruption that is predominately on the trunk or extremities.

Study drug-related TEAEs were reported for 84% of patients in the oral studies; 49% were Grade 3 or higher. Most were in the system organ classes of GI disorders (59% overall, 10% ≥Grade 3), general disorders (43% overall, 8% ≥Grade 3), blood and lymphatic system disorders (38% overall, 27% ≥Grade 3), and nervous system disorders (37% overall, 4% ≥Grade 3). The most common preferred terms included nausea (35% overall, 3% ≥Grade 3), diarrhea (32% overall, 6% ≥Grade 3), fatigue (28% overall, 6% ≥Grade 3), vomiting (25% overall, 3% ≥Grade 3), and thrombocytopenia (24% overall, 16% ≥Grade 3).

1.6 Dose Rationale

Patients will receive TAK-659 once daily for fifteen days and will receive Ixazomib capsules once weekly on days 1, 8, 15. The length treatment will be 15 days out of 21 days (one week rest).

In contrast with the continuous dosing of TAK-659 in C34002, in this combination study, cyclical administration will occur during only 15 days of a 21 day cycle (one week rest period). We anticipate this rest period will accommodate myeloid recovery following a period of intensive cytoreduction that has been noted when TAK-659 is combined with proteasome inhibitor in vitro (and as observed with a combination of proteasome inhibitor Bortezomib with Sorafenib in our recently completed trial).

The starting dose for the respective drugs in the Phase I portion of the current study will be directed by: a) a dose below the RP2D of TAK-659 (140mg po daily) from C34002, where TAK-659 at 100mg once daily will only be given 15 of the 21 days that have already been explored in its phase Ib trial (thus representing a dose and schedule reduction); and b) a two dose-level reduction (2.3mg) from the Ixazomib approved dose (4 mg) and given in a schedule of day 1, 8, 15 out of 21 days. As noted in the introductory section (and data not shown) we have extensive in vitro evidence for cytotoxicity of the relevant AML molecular genetic phenotypes, across a range of doses of these agents in combination.

Thus, in this trial we would be initially assessing tolerability at two dose levels below the approved 4 mg Ixazomib dose, but nevertheless where activity may be seen with the combination in Ixazomib run-in to escalation. Activity of the combination would be expected to appear early, within the 15 day treatment-timeframe for Ixazomib (and continued without another dose administration due to the prolonged half-life), as was previously demonstrated for cyclical administrations in our prior combination trial. Again, our prior study of drug combinations demonstrated that responses occur early, associated with profound blast cell cytoreduction in marrow and peripheral blood, and those responses observed were found to involve a recovery phase, for regrowth of mature differentiated cells sustaining hematopoiesis, in a cyclical fashion. This would be expected to occur without a possibility for differentiation syndrome that is known to describe the experience using a number of single agent TKIs. Therefore, toxicity of the combination will have been avoided by the cyclical administration schedule, while allowing for deep response to occur, and also avoiding differentiation syndrome, due to the earlier achievement active drug doses in the combination.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 Objectives

To perform Phase I and Phase II studies of the combination of TAK-659 with Ixazomib in order to assess the safety and tolerability of the combination (in phase I) and in the phase II to evaluate the preliminary efficacy of the combination.

2.1.1 Primary Objective

Phase I: To determine the maximum tolerated dose (MTD) and recommended phase 2 dose(s) (RP2D) of the combination of TAK-659 and Ixazomib in patients with relapsed or refractory AML.

Phase II: To evaluate preliminary efficacy of TAK-659 plus Ixazomib in relapsed or refractory AML as measured by overall response rate (ORR)

2.1.2 Secondary Objectives

- To evaluate the differential efficacy measures of TAK-659 with Ixazomib, involving presence or absence of Flt3 mutation(s), and/or mutational profile by NGS, additionally affecting epigenetic modifiers
- To evaluate additional efficacy measures of TAK-659 with Ixazomib, such as duration of response (DOR), time to progression (TTP), mortality rate at 3 and 6 months, and overall survival (OS)
- To evaluate the influence of multiple cycles of the combination on the outcome measures when applied in context of subsequent feasibility for bridge to transplantation as consolidation therapy
- To evaluate the safety and tolerability using NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.

2.1.3 Tertiary/Exploratory Objectives

- To measure the changes in TAK-659 plasma concentrations when given in combination with ixazomib.
- To quantitatively examine before, and at early timepoint of therapy, the primary mechanistic transcriptional drivers of these AMLs (HOXA and MEIS1), which are postulated to be extinguished by the drug combination, for their ability, when extinguished, to predict subsequent response
- To implicate Syk, Wnt pathway (ID1, JUN, FOXM1, β-catenin) and Wnt antagonists RUNX3 and Cullin 3 and EZH2 in responses affected by combination of TAK-659 and Ixazomib
- To use digital PCR technology to examine the participation of collaborating mutations on depth of remission for the class 1 and class 2 drivers

2.2 Endpoints

2.2.1 Primary Endpoint

Phase I: Maximum tolerated dose (MTD) and recommended Phase II dose (RP2D) for the combination of TAK-659 plus Ixazomib in relapsed or refractory AML

Phase II: Overall response rate (ORR) defined as CR, CRp, CRi, and PR per Revised Recommendations of the International Working Group (IWG) for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia.

2.2.2 Secondary Endpoints

- DOR defined as the time from the date of first documentation of a response to the date of first documented progressive disease
- TTP defined as the time from first dose of study drug until tumor progression as defined by the Revised Recommendations of the International Working Group (IWG) for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia
- Mortality rate at 3 and 6 months
- OS defined as the time from the date of study entry to death
- ORR, DOR, TTP, mortality rate at 3 and 6 months, and OS in Flt3mutant vs. WT populations

2.2.3 Tertiary/ Exploratory Endpoints

- Changes in TAK-659 plasma concentrations when given in combination with ixazomib.
- Identification of the cytogenetic and next-generation sequencing-derived mutational complement that predicts response to the combination therapy.
- Identification of a gene expression signature which identifies patients prior to therapy whom will achieve remission based on reversal of a pattern for repression of tumor suppressors, esp. RUNX3, and also achieve downregulation of pre-existing overexpressed HOXA and MEIS1, with participation in loss of Wnt pathway agonists (ID1, JUN, FOXM1, β-catenin).

3. ELIGIBILITY CRITERIA

3.1 Inclusion Criteria

Subject must meet all of the following applicable inclusion criteria to participate in this study:

1. Written informed consent and HIPAA authorization for release of personal health information. **NOTE:** HIPAA authorization may be included in the informed consent or obtained separately.
2. Male or female patients 18 years or older at the time of consent.
3. Patients must have a diagnosis of primary or secondary AML with relapsed or refractory disease (WHO update 2016, [34]) other than acute promyelocytic leukemia, or complex karyotype or monosomy 7 (or containing monosomy 7) for whom no standard therapies are anticipated to result in a durable remission according to the clinical judgment of the treating physician, or in a patient who refuses standard therapies. However, if the qualifying 3-5 complex karyotypic abnormalities entail recurring translocations/deletions normally of intermediate risk (5q- *or* 7q-, 11q23, MLL-PTD, t(3;21)(q26; q22), CBF+); and/or the mutational profile by NGS has revealed a profile which renders sensitivity to: 1) TAK-659 (DNMT3A; Flt3N676K, Flt3 S451F, in addition to the common Flt3ITD/TKD; combination of ASXL1 plus EZH2; or TET2 or IDH1/2) or to: 2) Ixazomib (NRAS,KRAS, PTPN11/SHP2, PDCD11, WT1), the patient will be eligible.
4. Must have submitted for Next Generation Sequencing (NGS) testing by: 1) (preferred) having submitted a tumor sample for commercial myeloid NGS from Foundation One, Mayo, Tempus, Quest, ARUP or an institutional CLIA-certified laboratory and/or 2) obtaining a bone marrow

aspirate during screening for submission to Foundation One, Mayo, Tempus, Quest, ARUP or an institutional CLIA-certified laboratory and ordered as standard of care. If bone marrow aspiration has failed, a peripheral blood sample with circulating blasts may be substituted. Screening reports on tumor cytogenetics and/or mutation assays (eg, FLT-3, and NGS) performed as part of the standard of care will be recorded in the study database or obtained at the time of screening if not previously available.

5. Patient's disease must be characterized for the presence/absence of Flt3ITD/TKD mutation from an FDA-approved vendor (ex. LabPMM).
6. In addition, patients for the phase 2 portion of the study must meet the following:
   a. Patients, if relapsed/ refractory, must have exposure to no more than 2 prior chemotherapy regimens. Re-induction with the same regimen or stem cell transplant will not be considered a separate regimen.
   b. Patients must not have prior exposure to any investigational Flt3 inhibitors (midostaurin or gilteritinib are allowed)
7. Eastern Cooperative Oncology Group (ECOG) performance status 0-2
8. Life expectancy of greater than 3 months as determined by the treating physician.
9. Female patients who:
   - Are postmenopausal for at least 1 year before the screening visit, OR
   - Are surgically sterile, OR
   - If they are of childbearing potential, agree to practice 2 effective methods of contraception at the same time, from the time of signing the informed consent through 180 days after the last dose of study drug, or
   - Agree to practice true abstinence, when is in line with the preferred and usual lifestyle of the subject. Periodic abstinence [eg, calendar, ovulation, symptothermal, postovulation methods], withdrawal, spermicides only, and lactational amenorrhea are not acceptable methods of contraception. Female and male condoms should not be used together.)
   - Agree not to donate eggs (ova) during the course of this study or 180 days after receiving their last dose of study drug.
10. Male patients, even if surgically sterilized (i.e., status post-vasectomy), who:
    - Agree to practice effective barrier contraception during the entire study treatment period and through 180 days after the last dose of study drug, or
    - Agree to practice true abstinence, when this is in line with the preferred and usual lifestyle of the subject. (Periodic abstinence [eg, calendar, ovulation, symptothermal, postovulation methods for the female partner] and withdrawal are not acceptable methods of contraception. Female and male condoms should not be used together.)
    - Agree not to donate sperm during the course of this study or within 180 days after receiving their last dose of study drug.
11. Voluntary written consent must be given before performance of any study related procedure not part of standard medical care, with the understanding that consent may be withdrawn by the patient at any time without prejudice to future medical care
12. In the absence of rapid progressive disease, the interval from prior systemic anticancer treatment to time of TAK-659/Ixazomib administration should be at least 2 weeks for cytotoxic agents

(other than hydroxyurea), or at least 5 half-lives for noncytotoxic agents, and patients have to have recovered from acute toxicities of these therapies. Patients who are on hydroxyurea may be included in the study and may continue on hydroxyurea for the first 21 days while participating in this study. **NOTE:** For patients with a white blood cell count >50,000/uL, hydroxyurea may be used to control the level of circulating leukemic blast cell counts prior to study entry, and, if needed, concomitantly while on TAK-659 treatment during the first 21 days of the study.

13. Suitable venous access for the study-required blood sampling, and transfusion support.
14. Demonstrate adequate organ function as defined in the table below; all screening labs to be obtained within 28 days prior to registration.

System	Laboratory Value
Renal	
Calculated creatinine clearance	≥ 60 mL/min using the Cockcroft-Gault formula
Pancreas	
Lipase	≤1.5xULN with no clinical symptoms suggestive of pancreatitis or cholecystitis
Amylase	
Hepatic	
Total Bilirubin	≤ 1.5 × upper limit of normal (ULN)
Aspartate aminotransferase (AST)	≤ 2.5 × ULN
Alanine aminotransferase (ALT)	≤ 2.5 × ULN

3.2 Exclusion Criteria

Subjects meeting any of the criteria below may not participate in the study:

1. Clinically active central nervous system leukemia.
2. Female patients who are lactating and breastfeeding (**NOTE:** breast milk cannot be stored for future use while the mother is being treated on study)
3. Female patients who have a positive serum pregnancy test during the screening period or a positive urine pregnancy test on Day 1 before first dose of study drug.
4. Any serious medical or psychiatric illness, including drug or alcohol abuse, that could, in the investigator's opinion, potentially jeopardize the safety of the patient or interfere with the objectives of the study.
5. Prior treatment with investigational agents ≤ 21 days or ≤ 5 half-lives (whichever is shorter) before the first dose of study treatment. A minimum of 10 days should elapse from prior investigational therapy to initiating protocol therapy.
6. Persistent clinically significant toxicity from prior chemotherapy that is Grade 2 or higher by the NCI CTCAE (v5).
7. Receipt of HSCT within 60 days of the first dose of TAK-659/Ixazomib; clinically significant graft-versus-host disease (GVHD) requiring ongoing immunosuppressive therapy post HSCT at the time of screening (use of topical steroids for ongoing skin GVHD is permitted).
8. Active, systemic infection requiring intravenous (IV) antibiotic, antifungal, or antiviral therapy or other serious infection within 10 days before the first dose of study drug.

9. Major surgery within 14 days before the first dose of study drug and have not recovered fully from any complications from surgery.
10. Radiotherapy less than 2 weeks before the first dose of study treatment or have not recovered from acute toxic effects from radiotherapy.
11. Known human immunodeficiency virus (HIV) positive.
12. Known hepatitis B surface antigen positive, or known or suspected active hepatitis C infection (testing not required).
13. Any of the following cardiovascular conditions:
    - Acute myocardial infarction within 6 months before starting study drug.
    - Current or history of New York Heart Association Class III or IV heart failure.
    - Evidence of current, uncontrolled cardiovascular conditions including cardiac arrhythmias, angina, pulmonary hypertension, or electrocardiographic evidence of acute ischemia or active conduction system abnormalities.
    - Fridericia corrected QT interval (QTcF) >450 milliseconds (msec) (men) or >475 msec (women) on a 12-lead ECG during the Screening period.
    - Abnormalities on 12-lead ECG including, but not limited to, changes in rhythm and intervals that, in the opinion of the treating physician, are considered to be clinically significant.
14. Known gastrointestinal (GI) disease or GI procedure that could interfere with the oral absorption or tolerance to study drugs, including difficulty swallowing tablets, diarrhea > Grade I despite supportive therapy.
15. Use or consumption of any of the following medications, supplements, or foods/beverages that are inhibitors or inducers of P-gp or strong reversible inhibitors or inducers of CYP3A within the indicated timeframes below. Note that use or consumption of these substances is not permitted during the study.
    a) Inhibitors of P-gp and/or strong reversible inhibitors of CYP3A within 5 times the inhibitor half-life (if a reasonable half-life estimate is known), or within 7 days (if a reasonable half-life estimate is unknown), before the first dose of study drug. <u>In general, the use of these agents is not permitted during the study except in cases where an AE must be managed.</u> See Section 5.7.2 and the Documents/Info tab of the electronic data capture (EDC) system for a nonexhaustive list of strong CYP3A reversible inhibitors and/ or Pgp inhibitors based on the FDA Draft DDI Guidance.
    b) Strong CYP3A mechanism-based inhibitors or strong CYP3A inducers and/or P-gp inducers within 7 days, or within 5 times the inhibitor or inducer half-life (whichever is longer), before the first dose of study drug. However, if a patient has a strong indication for fungal prophylaxis, then the moderate CYP3A inhibitors fluconazole or isovuconazole (mold-active) is recommended for prophylaxis, and the TAK-659 dose should be reduced to 60mg, along with increased scrutiny for toxicity. Similarly, if the patient develops a fungal infection during a productive intervention with TAK-659/Ixazomib combination, isovuconazole (mold-active) should be used, along with increased scrutiny for toxicity. In general, the use of these agents is not recommended during the study except in cases where such an AE must be managed, and the intermittent schedule of TAK-659/Ixazomib combination will not prevent profound neutropenia. See Section 5.7.2 and the Documents/Info tab of the EDC for

a non-exhaustive list of strong CYP3A mechanism-based inhibitors or strong CYP3A inducers and/or P-gp inducers based on the FDA Draft DDI Guidance.

c) Grapefruit-containing food or beverages within 5 days before the first dose of study drug.

16. Lack of suitable venous access for the study-required blood sampling.
17. Diagnosed or treated for another malignancy within 2 years before study enrollment or previously diagnosed with another malignancy and have any evidence of residual disease. Patients with nonmelanoma skin cancer or carcinoma *in situ* of any type are not excluded if they have undergone complete resection.
18. Patient has ≥ Grade 3 peripheral neuropathy, or Grade 2 with pain on clinical examination during the screening period.
19. Known allergy to any of the study medications, their analogues, or excipients in the various formulations of any agent.

4. SUBJECT REGISTRATION

All subjects must be registered through Big Ten Cancer Research Consortium (Big Ten CRC) Administrative Headquarters'(AHQ) electronic data capture (EDC) system.

Subjects must be registered prior to starting protocol therapy. Subjects should begin therapy within 5 business days of registration. However, beginning therapy outside that timeframe will not be considered a deviation.

5. TREATMENT PLAN

This study is an open-label, multicenter, phase I dose escalation and phase IIa study of TAK-659 in combination with Ixazomib, in adult patients with relapsed or refractory AML. This study will include a phase I portion for evaluating the safety of the TAK-659 and Ixazomib, along with monitoring for early efficacy signal, and a single-arm phase II portion. It is expected that up to 18 patients will be enrolled in the phase I portion and up to 36 patients will be enrolled in the phase II portion of this study.

Once enrolled, patients will be given TAK-659 tablets and Ixazomib capsules, once daily for fifteen days (TAK-659) and once weekly (Ixazomib) on days 1, 8, 15, respectively. The cycle length will be 15 days of treatment out of 21 days (one week rest).

To determine the MTD of the combination, a 3+ 3 approach to dose escalation design will be used. Prior to commencing dose/schedule advance, patients in the immediately preceding cohort will have completed two full cycles of the regimen and will have been evaluated for toxicity. Next, Ixazomib dose escalation will be undertaken (in context of the 15-day treatment cycle/21-day complete cycle including 1 week rest schedule).

At least 6 patients will be evaluated at RP2D (either at MTD or at lower doses as determined) before making a decision to advance to the phase 2 study. In the process of determining or refining RP2D, expansion of more than 1 dose level to at least 6 additional patients is permissible so that pharmacodynamic measures and early signs of clinical activity can be assessed to assist dose selection.

AEs will be assessed, and laboratory values, vital signs, and ECGs will be obtained to evaluate the safety and tolerability of the combination. Toxicity will be evaluated according to NCI Common Terminology Criteria for Adverse Events (NCI CTCAE), version 5. Dose-limiting toxicities (DLTs) are defined in Section 5.5.

Patients will be treated until either disease progression or occurrence of unacceptable study drug-related toxicities. Patients may discontinue therapy at any time. Patients will attend the safety follow up visit 30 days (+10 days) after receiving their last dose of study drug or before the start of subsequent anticancer therapy (other than hydroxyurea) if that occurs sooner.

Assessment of disease response will follow the criteria outlined in the Revised Recommendations of the International Working Group (IWG) for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia.

Pharmacodynamic activity of the combination regimen will be assessed in Phase I and Phase II by evaluating bone marrow AML blasts at the time-points specified in the study calendar. Bone marrow biopsies and aspirates for disease response monitoring will be obtained as described in the study calendar. Residual and/or designate samples from bone marrow biopsies and aspirates collected for disease assessment will also be used for analysis of biomarkers as specified in the study calendar.

5.1 Number of Patients

For the phase I portion, up to 18 patients will be enrolled in this study from approximately 2 to 4 study centers in the US. For the phase II portion, 36 patients will be enrolled from these centers.

Patients who are withdrawn from treatment during Cycle 1 for reasons other than toxicity during the phase I portion of the study will be replaced. Patients in the phase II portion of the study, beyond the Simon's two-stage threshold, who are not evaluable for response will be replaced; however, patients who receive at least 1 dose of study drugs, and have measurable disease on bone marrow exam or by biopsy-documented extramedullary tumor at baseline, will be used for futility analyses, including the Simon's two-stage response evaluation.

Duration of Study

Patients, including those who achieve a CR, may receive the combination until they experience disease progression. Patients will discontinue treatment if they have an unacceptable drug-related toxicity. The maximum duration of treatment, however, will be 12 months from Cycle 1 Day 1 (C1D1) unless it is determined that a patient would derive benefit from continued therapy beyond 12 months.

Patients will be followed for 30 days after the last dose of the combination, or to the start of subsequent anticancer therapy, whichever occurs first, to permit the detection of any delayed treatment-related AEs. All patients, including those patients no longer on treatment, will be assessed for survival. Patients who discontinue the study, regardless of reasons for discontinuation, will be followed for up to 12 months after discontinuation of the study drug for survival (every month until death), loss to follow-up, or withdrawal of consent for further follow-up. In addition, information on any subsequent anticancer therapies will be collected during the survival follow-up period. For patients who achieve CR but discontinue study treatment while still in remission, disease progression based upon available local data will also be collected during the survival follow-up period.

The final analyses for the clinical study report may be conducted after all patients enrolled in the study have had the opportunity to complete 3-4 cycles of combination therapy (while still not progressing).

It is anticipated that this study will last for approximately 24-32 months, including 9 to 12 months in the phase I portion, and 15 to 20 months in the phase II portion.

5.2 Phase Ib Dose Escalation

Dose escalation will involve only Ixazomib, increasing from 2.3 mg and then to 3 mg. Patients in each cohort will continue to the completion of the second cycle, and undergo evaluation of toxicity/tolerability prior to consideration of dose-escalation to the next cohort. The maximal dose of Ixazomib at 4mg was determined as in aforementioned myeloma studies.

Dose Cohort	**Number of patients**	**TAK-659 (PO)** Days 1-15	**Ixazomib (PO)** Days 1, 8, 15
1	3-6	100 mg, once daily	2.3 mg
2	3-6	100 mg, once daily	3 mg
3	3-6	100 mg, once daily	4 mg

Patients within each cohort will be followed for toxicity occurring within the first 2 cycles prior to initiating a dose/schedule escalation of the individual drugs in the next cohort, in context of the type of dose limiting toxicity (DLT) observed and to what study drug(s) the toxicity is related.

In cohort 1, If 1/3 patients develop grade ≥ 3 DLT toxicity, then 3 more patients will be entered at this dosing level; if < 2/6 patients develop DLT, then the next cohort will involve escalation of Ixazomib in dose.

If, on the other hand, toxicity attributable to the combination is seen in the first cohort at ≥ 2 patients, then we will consider modification of the protocol to reduce the initial dose of TAK-659 and/or Ixazomib. Again, our prior experience involving the addition of pharmacodynamically-active dosing as starting to a combination of Sorafenib and Vorinostat suggest we will not see this reduction as necessary. The dose modification plan will be based on the nature of the DLT:

Dose Cohort	**Number of patients**	**TAK-659* (PO)** Days 1-15	**Ixazomib* (PO)** Days 1, 8, 15
1	3-6	100 mg, once daily	2.3 mg
[redacted]			
Dose modification plan			
[redacted]		80 mg, once daily	2.3 mg Contact sponsor-investigator for consideration of schedule below 2.3mg.
*On clinic days for PK determination, TAK-659 will be taken first and then Ixazomib.			

In cohort 2, if 1/3 patients develop grade ≥ 3 DLT toxicity, then 3 more patients will be entered at this dosing level; if < 2/6 patients develop DLT, then the next cohort will involve escalation of Ixazomib in dose.

Dose Cohort	**Number of patients**	**TAK-659* (PO)** Days 1-15	**Ixazomib* (PO)** Days 1, 8, 15
2	3-6	100 mg, once daily	3 mg
[redacted]			
Dose modification plan based on the nature of DLT			
If DLT at least possibly related to TAK-659 **OR** not related to Ixazomib			
[redacted]		80 mg, once daily	3 mg
If DLT at least possibly related to Ixazomib **OR** not related to TAK-659			
[redacted]		100 mg, once daily	2.3 mg
If DLT at least possibly related to TAK-659 **AND** Ixazomib			
[redacted]		80 mg, once daily	2.3 mg
*On clinic days for PK determination, TAK-659 will be taken first and then Ixazomib.			

In cohort 3, if ≥2/6 patients develop DLT, a consultation will be made between the sponsor and investigators: either the previous dose level will be considered the MTD (if 6 or more patients have been studied at that dose level), the previous dose level will be expanded (if fewer than 6 patients have been studied at that dose level), or we will modify the the protocol to add a dose level intermediate between the current and the previous dose level.

Dose Cohort	**Number of patients**	**TAK-659* (PO)** Days 1-15	**Ixazomib* (PO)** Days 1, 8, 15
3	3-6	100 mg, once daily	4 mg
[redacted]			
Dose modification plan based on the nature of DLT			
If DLT at least possibly related to TAK-659 **OR** not related to Ixazomib			
[redacted]		80 mg, once daily	4 mg
If DLT at least possibly related to Ixazomib **OR** not related to TAK-659			
[redacted]		100 mg, once daily	3 mg
If DLT at least possibly related to TAK-659 **AND** Ixazomib			
[redacted]		80 mg, once daily	3 mg
*On clinic days for PK determination, TAK-659 will be taken first and then Ixazomib.			

As in usual protocol for MTD/RP2D determination, dose escalation will continue until these endpoints are determined based on safety, tolerability, PD, and preliminary efficacy data, if available. We also realize that if ≥ 2 patients experience DLT in each of the first two cohorts at founding TAK-659 dose used in the 3+3 escalation, then one (half) dose reduction step of TAK-659 will be needed.

5.2.1 TAK-659 Dose Accomodation with required concomitant dosing anti-fungal agent as moderate activity CYP3A inhibitor

Despite the institution of a more favorable intermittent schedule of the combination of TAK-659 with Ixazomib presented here, when compared to continuous single agent TKI, AML is an intrinsic cause of primary neutropenia leading to fungal infections. Patients considered to have high risk for the development of fungal infection, or with a prior history of an aspergillus/mold infection, now recovered, may require concomitant administration of a moderate CYP3A inhibitor such as fluconazole, or, in the case of the latter ongoing infection more likely to involve aspergillus/mold, isovuconazole. Similarly, patients whom are being treated with benefit, on the existing dose level of the combination, may develop a fungal infection and require isovuconazole.

Patients requiring prophylaxis with fluconazole or patients whom develop active fungal infection, where isovuconazole would be appropriate, continuation of TAK-659 in the combination will require dose reduction to 60mg po daily of the TKI, and careful analysis of toxicity parameters, especially including QTc and hepatic and pancreatic enzymes. Dosing of Ixazomib will not require modification in the absence of relatable toxicity. The above determined TAK-659 dose cannot be escalated. If toxicity supervenes, the patient will be taken off study for alternative anti-infective therapy as needed. Should no toxicity be observed in a cohort, stepwise dose escalation, first to 80mg, may be considered based on review of concurrent PK measurements of specific antifungal impact on TAK-659 levels.

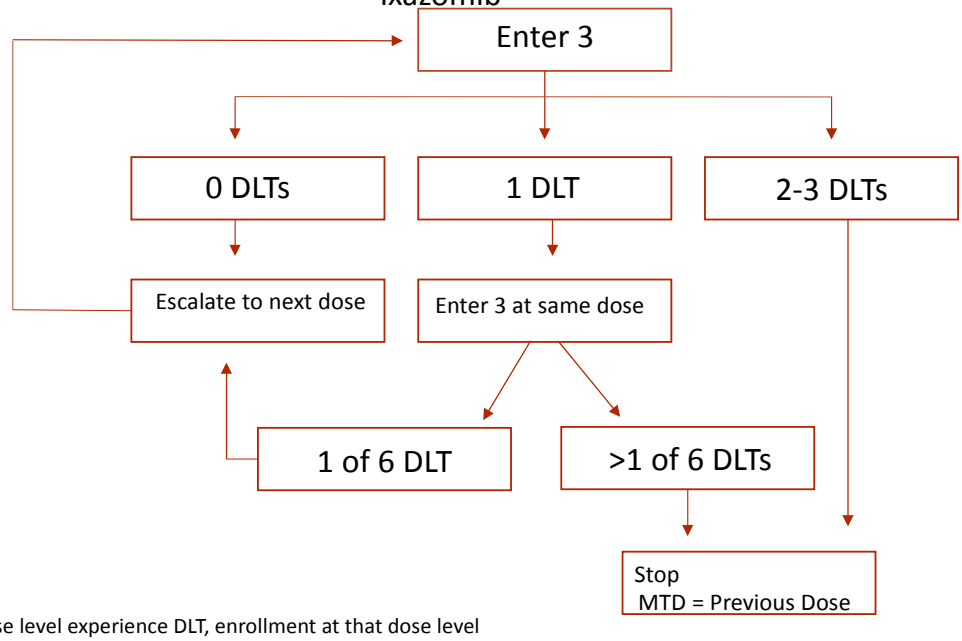
5.2.2 Dose Reduction or Discontinuation in the setting of Pneumonitis

Should the patient develop grade 1 or 2 pneumonitis, the combination therapy will be held until the episode resolves and the TAK-659 and Ixazomib doses will undergo 1-step reductions, respectively. If the episode recurs the patient will be taken off study. Similarly, should the patient develop grade 3+ pneumonitis, permanent drug discontinuation will be required.

5.3 Dose Escalation Rules and MTD Definition

Dose escalation will be determined based on the number of toxicities as noted by the algorithm noted in the following Figure 5-1.

Figure 5-1 Dose/Schedule Escalation Algorithm TAK-659 and Ixazomib



TAK-659/Ixazomib
Clinical Study Protocol

If 2 or more patients in any dose level experience DLT, enrollment at that dose level will stop. Following consultation between the sponsor and investigators, either the previous dose level will be considered the MTD (if 6 or more patients have been studied at that dose level), the previous dose level will be expanded (if fewer than 6 patients have been studied at that dose level), or a dose level intermediate between the current and the previous dose level will be evaluated.

To define the Maximum Tolerated Dose (MTD), subjects will be evaluated for DLT following two cycles (56 days) of treatment. Decisions to move to next dose escalation cohort will not be made until all subjects complete 2 cycles of therapy at a given dose.

The MTD is defined as the dose level at which fewer than 33% of subjects experience a dose limiting toxicity (DLT), and specifically is the dose level at which less than 2 out of 6 subjects experience DLT. The minimal safe and effective dosing with pharmacodynamic and pharmacokinetic parameters pointing toward the goal of efficacy optimization, will be the recommended dose (RP2D) for the Phase II component of the study. To achieve this, the developing results of safety, tolerability, efficacy, PK and PD in each dose-escalation cohort will be reviewed in real time after each cohort, to determine whether maximal safety and evolving efficacy milestones will have been reached based on examination of two consecutive cohorts, when safety and tolerability measures are within criteria for each.

- The criterion for safety will be DLT <20% within the cohort.
- The criterion for tolerability will be the administration of >80% of expected dose within the cohort.
- The criterion for efficacy will be the dose with an estimation of greater or equal to 25% ORR in the Flt3 mutant cohort, or greater than or equal to 10% ORR in the Flt3 wild-type cohort.
- The criterion for optimizing PK will be: on day 1, equal or greater than 100ng/ml peak (Cmax) TAK-659, or its trough equal or greater than 15ng/ml; on day 15, peak at least 200ng/ml, trough at least 40ng/ml. On the other hand, the day 15 peak should be less than 400ng/ml, and trough should be less than or equal to 100ng/ml.

- The criteria for defining an optimization of PD will be the reduction in more than one of the epigenetic drivers by 50%: JUN, ID1, β-catenin, HOXA 9/10, MEIS1 or rise by 50% of the epigenetic repressor RUNX3.

Subjects will initially be enrolled onto dose level 1. Three to six evaluable subjects will be enrolled at each dose level. All subjects assigned to a dose level must be followed for at least 2 cycles before dose escalation to the next cohort level can begin.

Note: Subjects not evaluable for DLT assessment (i.e. do not complete at least 1 cycle of therapy due to reasons other than toxicity) on the Phase I portion of the protocol will be replaced for determination of dose escalation.

The following rules will be followed:

- An initial three subjects will be enrolled in cohort 1. If all 3 subjects in cohort 1 complete 2 cycles of therapy without dose limiting toxicity (DLT), the study will proceed to enroll 3 subjects at cohort 2. If all 3 subjects in cohort 2 complete 2 cycles of therapy without DLT, the study will proceed to Phase II with the MTD of each drug established.
- Alternatively, if 1 of the first 3 subjects in any given dose cohort experiences DLT, an additional 3 subjects will be enrolled at that dose level. If only 1 of the total 6 subjects in a dose level experience DLT, the study will proceed to the next dose level as planned. If 2 of the total 6 subjects in any given dose cohort experience DLT, the next lower dose level will be explored and considered the maximum tolerated dose (MTD) if no more than 1 of 6 subjects experience a DLT. That dose will be recommended for the Phase II study.

Number of subjects with DLT at given dose level	**Escalation decision**
0 out of 3	Enter 3 subjects at the next dose level.
≥2 out of 3	Dose escalation will be stopped. This dose level will be declared the **maximum administered dose**. Three (3) additional subjects will be entered at the next lower dose level if only 3 subjects were treated previously at that next lower dose level.
1 out of 3	Enter at least 3 more subjects at this dose level: • If 0 of these additional 3 subjects experience DLT, proceed to the next dose level. • If 1 or more of these additional 3 subjects experience DLT, then dose escalation is stopped and this dose is declared the maximum administered dose. Three (3) additional subjects will be entered at the next lower dose level if only 3 subjects were treated previously at that dose.
≤1 out of 6 at highest dose level below the maximum administered dose	This will be defined as the MTD. This dose level will be the recommended Phase II dose.

5.3.1 Number of treatment cycles

Given our experience with this sort of treatment algorithm, we will follow the cues previously established in the Sorafenib/Vorinostat/Bortezomib trial to determine the course.

We will follow the peripheral blood blast reduction during the first cycle of therapy. When peripheral blasts have been reduced by at least 50% , we will perform a bone marrow, either day 4 or 8, to document if similar effects are occurring in the marrow as in the periphery. Also, excess cells obtained from the marrow at this early time point will be used to inform the impact of changes in gene signature from baseline marrow, which will have been obtained on the registration/pretreatment visit at day -4 to 0.

Usual supportive care transfusions will occur during the treatment cycle to maintain platelet count >10k at weekly assessments, and hematopoietic growth factors are not mandated as long as ANC >200/uL. Treatment outcome assessment will involve a bone marrow performed at day 15.

Assuming achievement of 50% or greater reduction in marrow blast score (cellularity x blast percentage) after 2 cycles, another cycle will be given. If the patient has had a response (CR, CRp or CRi) after 2 cycles, every attempt will be made to transition the patient to transplant.

However, if the patient is not a transplant candidate but the response after 2 cycles reaches the threshold 50% reduction, continuation of therapy cycles can continue with follow up bone marrow after the next 2 cycles as long as no evidence of peripheral progression occurs. Evidence for progression in the bone marrow would be a cue for discontinuation of therapy.

5.4 Definition of Dose Limiting Toxicity

Toxicity will be evaluated according to the NCI CTCAE version 5.

DLT is defined as any of the following events occurring within the first 2 cycles that are considered by the treating physician to be at least possibly related to therapy with study drug(s). AEs in which the relationship to study drug cannot be ruled out should be considered possibly related to study drugs TAK-659 and/or Ixazomib.

- Prolonged myelosuppression with the persistence of ≥ Grade 4 neutropenia or thrombocytopenia in the absence of leukemia (blast count <5% in bone marrow) at least 42 days after the initiation of therapy.
- Any Grade 3 or greater nonhematologic toxicity with the following exceptions:
  - Grade 3 nausea and/or emesis resolved to ≤ Grade 1 or baseline in a week after the use of an optimal antiemetic regimen based on standard practice. The optimal antiemetic regimen is defined as an antiemetic regimen that employs both a 5-hydroxytryptamine 3 serotonin (5-HT3) antagonist and a corticosteroid given in standard doses and according to standard schedules. Grade 3 nausea, vomiting must not require tube feeding, total parenteral nutrition, or require prolonged hospitalization.
  - Grade 3 diarrhea that resolved to ≤ Grade 1 or baseline in a week after receiving the maximal supportive therapy based on standard practice. Grade 3 diarrhea must not require tube feeding, total parenteral nutrition, or require prolonged hospitalization.
  - Brief (<1 week) Grade 3 fatigue.
  - Electrolyte abnormalities correctable within 72 hours

- Grade 3 or 4 tumor lysis syndrome if it is successfully managed clinically and resolves within 7 days without end-organ damage.

- Failure to administer ≥ 75% of planned doses of the study drug due to TAK-659- or Ixazomib-related or possibly related hematologic (considered not related to leukemic infiltration) or nonhematologic toxicities.
- Other TAK-659- or Ixazomib-related Grade 2 or greater nonhematologic toxicities that, in the opinion of the investigator, require a dose reduction or discontinuation of therapy.
- Confirmed Hy's law/guideline cases of drug-induced liver injury: ALT or AST >3× upper limit of normal and total bilirubin >2× ULN, which is not cholestatic (ALT value ÷ ALT ULN] ÷ [Alkaline phosphatase value ÷ Alk phos ULN]= <2), and not caused by disease. This will require permanent drug discontinuation.
- Any confirmed grade 4 elevation of a single liver enzyme, even absent bilirubin elevation, will require permanent discontinuation from protocol therapy.

Although DLT-like events may occur at any point during treatment, DLTs defined during Cycles 1-2 of treatment will be given more influence for decisions regarding dose escalation for cohorts, expansion of a dose level, or evaluation of intermediate dose levels for purposes of MTD determination. Patients will be monitored through all cycles of therapy for treatment-related toxicities.

5.5 Phase II Study

Upon enrollment, the mutational profiles from Foundation One, Mayo, Tempus, Quest, ARUP or an institutional CLIA-certified laboratory will be recorded in the study database with minimum gene coverage as noted in Section 8.3. Subjects will be enrolled into either the FLT-3 mutant cohort or the FLT-3 WT cohort according to their mutation assay results.

5.6 TAK-659 and Ixazomib Administration

Drug	Dose	Route[1]	Schedule[2]	Cycle Length
TAK-659	RP2D	PO	Days 1-15	21days
Ixazomib	RP2D	PO	Days 1, 8, 15	
1: Please see details below regarding administration of oral study drugs. On clinic days for PK determination, TAK-659 will be taken first and then Ixazomib. N**OTE:** Patients will be asked to keep a diary of dosing and bring to each clinic visit. **2**: A window of ± 3 days may be applied to all study visits to accommodate observed holidays, inclement weather, scheduling conflicts etc. Date and time of each drug administration should be clearly documented in subject's chart and electronic case report forms (eCRFs).				

5.6.1 TAK-659 Administration

TAK-659, should be taken on an empty stomach at least 1 hour before and no sooner than 2 hours after ingestion of food and/or beverages other than water. Each tablet should be swallowed separately with a sip of water. A total of approximately 8 ounces (240 mL) of water should be taken with the prescribed doses of TAK-659. Patients must swallow the tablets whole; the tablets must not be chewed, crushed, or manipulated in any way before swallowing.

Patients should be instructed to take their study medication at approximately the same time each day and to not take more than the prescribed dose at any time. If a patient fails to take TAK-659 one day, or if a patient does not take TAK-659 at their scheduled dosing time (± 6 hours of the scheduled dosing time), that dose should be skipped, and the patient must not make dose adjustments to account for the missed dose on subsequent days, for example, by taking a double dose of study drug(s) on the following day. Patients should record any skipped doses in their dosing diary (and resume dosing at the next scheduled time with the prescribed dosage.

If severe emesis prevents the patient from taking a TAK-659 dose, that dose will be skipped. If emesis occurs after TAK-659 ingestion, patients should not re-dose following emesis and should record the time of the emesis in their dosing diary. Patients should resume dosing at the next scheduled time with the prescribed dosage.

On clinic visit days, patients should be instructed to hold their dose until predose assessments are performed. The timing of the visit should be planned so that the pre-dose PK draw occurs near the same time as the previous day's dosing. TAK-659 administration guidelines described above will still be followed on clinic days. On clinic days for PK determination, TAK-659 will be taken first and then Ixazomib. Patients should be instructed to return their empty blister packs to the clinic, rather than discarding them. Reconciliation will occur accordingly when the patient returns for their next cycle of take-home medication.

5.6.2 Ixazomib Administration

Ixazomib should be taken once a week on the same day and at approximately the same time for the escalating cycle. Ixazomib should be taken at least one hour before or at least two hours after food. The whole capsule should be swallowed with water. A total of approximately 8 ounces (240 mL) of water should be taken with the capsules. Patients should be instructed to store the medication between +2°C and +30°C (35.6°F-86°F) for the duration of each cycle. Do not freeze Ixazomib or store above 30°C. Any extreme in temperature should be reported as an excursion and should be dealt with on a case-by-case basis.

The capsule should not be crushed, chewed or opened.

If an Ixazomib dose is delayed or missed, the dose should be taken only if the next scheduled dose is ≥ 72 hours away. A missed dose should not be taken within 72 hours of the next scheduled dose. A double dose should not be taken to make up for the missed dose. If the patient vomits after taking a dose, the patient should not repeat the dose but should resume dosing at the time of the next scheduled dose. On clinic visit days, patients should be instructed to hold their dose until pre-dose assessments are performed. Ixazomib administration guidelines described above will still be followed on clinic days. On clinic days for PK determination, TAK-659 will be taken first and then Ixazomib.

5.7 Concomitant Medications

During the study, patients will be instructed not to take any additional medications (including over-the-counter products and supplements) without prior consultation with the investigator. At each visit, the investigator will ask the patient about any new medications he/she is taking or has taken while on study. All concomitant medications (defined as any medication given during the study) and significant nondrug therapies, including physical therapy and blood transfusions, should be recorded from signing of the informed consent form (ICF) through 30 days after the last dose of study drug or the start of subsequent antineoplastic therapy, whichever occurs first.

5.7.1 Allowed Concomitant Medications

All treatments considered necessary for a subject's welfare may be administered at the discretion of the treating physician in keeping with the community standards of medical care.

5.7.2 Prohibited Concomitant Medications

The following restrictions apply during the study:

- Any antineoplastic therapy other than TAK-659 is prohibited during the study (with the exception of hydroxyurea for the first 21 days of study drug treatment). This includes chronic use of corticosteroids at daily doses greater than the equivalent of 10 mg of prednisone as part of any anticancer treatment regimens. If alternative therapy is required for treatment of the patient's tumor, the patient should be removed from this study and the reason for removal recorded in the electronic case report form (eCRF).
- Radiation therapy (note that, in general, the requirement for local radiation therapy indicates disease progression). Palliative radiotherapy for local pain/symptom control in a preexisting nontarget lesion may be considered.
- Prophylactic use of myeloid growth factors (eg, granulocyte colony stimulating factor [G-CSF], granulocyte macrophage-colony stimulating factor [GM-CSF]) in Cycle 1 during dose escalation. Patients who experience severe and/or febrile neutropenia in Cycle 1 of dose escalation can be managed with growth factor support if needed in accordance with American Society of Clinical Oncology (ASCO) guidelines.
- Concurrent systemic administration of TAK-659 with inhibitors or inducers of P-gp or strong inhibitors or inducers of CYP3A should be avoided in this study. In vitro studies indicate that TAK-659 is a substrate for P-gp and that, among CYP isozymes, TAK-659 is preferentially metabolized by CYP3A4/5. Refer to the list below and in the Documents/Info tab of the EDC for a nonexhaustive list of medications, supplements, and food products that are inhibitors or inducers of P-gp or strong inhibitors or inducers of CYP3A based on the US FDA draft guidance for DDI studies.
    - Antifungals: itraconazole, ketoconazole, posaconazole, voriconazole.
    - Antibiotics: azithromycin, clarithromycin, erythromycin, telithromycin.
    - Antimycobacterials: rifabutin, rifampin, rifapentine.
    - Antiepileptics: carbamazepine, phenobarbital, phenytoin, primidone.
    - Antidepressant: nefazodone.
    - Immunosuppressant: cyclosporine.
    - Calcium channel blockers: diltiazem, felodipine, mibefradil, verapamil.
    - Antiarrhythmics: amiodarone, dronedarone, quinidine.
    - Antiplatelet: ticagrelor.
    - Antilipid: avasimibe.
    - Other cardiovascular: captopril, carvedilol, ranolazine.
    - Vasopressin antagonist: conivaptan.
    - Food/herbals/supplements: grapefruit-containing food and beverages, St. John's wort, quercetin.

If a patient experiences an AE on study and TAK-659 dosing is temporarily interrupted because of that AE, the medications listed above and in the Documents/Info tab of the EDC may be used for AE management if there is no appropriate alternative treatment available per the treating physician's judgment and the dosing is not concurrent with study drug. This situation should be evaluated by the sponsor investigator and patients should be closely monitored for potential toxicities.

Please note that strong CYP3A inducers should not be administered with Ixazomib. Among those are rifampin, rifapentine, rifabutin, carbamazepine, phenytoin, phenobarbital, and St. John's Wort.

Note that medications used to treat HIV or hepatitis C infection are not listed above because patients with known HIV infection or known hepatitis C infection are excluded from study participation.

5.7.3 Precautions and Restrictions

Patients should not drive, operate dangerous tools or machinery, or engage in any other potentially hazardous activity that requires full alertness and coordination if they experience sedation while enrolled in this study.

Patients are to be instructed to limit the use of alcohol while enrolled in this study.

- Fluid deficit should be corrected before initiation of treatment and during treatment.
- Nonsteroidal anti-inflammatory drugs (NSAIDS) should be avoided with impaired renal function given reported NSAID-induced renal failure in patients with decreased renal function

5.8 Reproductive Considerations

It is not known what effects TAK-659 has on human pregnancy or development of the embryo or fetus. Therefore, female patients participating in this study should avoid becoming pregnant, and male patients should avoid impregnating a female partner. Nonsterilized female patients of reproductive age group and male patients should use effective methods of contraception through defined periods during and after study treatment as specified below.

Female patients must meet 1 of the following:

- Postmenopausal for at least 1 year before the screening visit, or
- Surgically sterile, or
- If they are of childbearing potential, agree to practice 1 highly effective methods of contraception and 1 additional effective (barrier) method, at the same time, from the time of signing of the informed consent form through 180 days(or longer, as mandated by local labeling [eg, USPI, SmPC, etc;]) after the last dose of study drug, or
- Agree to practice true abstinence, when this is in line with the preferred and usual lifestyle of the patient. (Periodic abstinence [eg, calendar, ovulation, symptothermal, postovulation methods], withdrawal, spermicides only, and lactational amenorrhea are not acceptable methods of contraception. Female and male condoms should not be used together.)
- Agree to not donate eggs (ova) during the course of this study or 180 days after receiving their last dose of study drug(s).

Male patients, even if surgically sterilized (i.e., status post-vasectomy) must agree to 1 of the following:

- Agree to practice highly effective barrier contraception during the entire study treatment period and through 180 days after the last dose of study drug, OR
- Agree to practice true abstinence, when this is in line with the preferred and usual lifestyle of the patient. (Periodic abstinence [eg, calendar, ovulation, symptothermal, postovulation methods for the female partner], withdrawal, spermicides only, and lactational amenorrhea are not acceptable methods of contraception. Female and male condoms should not be used together.)
- Agree not to donate sperm during the course of this study or 180 days after receiving their last dose of study drug.

Acceptable non-hormonal birth control methods:

- Total sexual abstinence ie, refrain from any form of sexual intercourse in line with the patients' usual and/or preferred lifestyle. Abstinence must be for the total duration of the study treatment and for at least 1 month (for female patients) or 3 months (for male patients) after the last dose of study treatment.
- NOTE: Periodic abstinence (eg, calendar ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.
- Vasectomised sexual partner PLUS male condom. With participant assurance that partner received post-vasectomy confirmation of azoospermia.
- Tubal occlusion PLUS male condom
- Intrauterine Device PLUS male condom. Provided coils are copper-banded.

Acceptable hormonal methods:

- Etonogestrel implants (eg, Implanon®, Norplant®) PLUS male condom
- Normal and low dose combined oral pills PLUS male condom
- Hormonal shot or injection (eg, Depo-Provera) PLUS male condom
- Intrauterine system device (eg, levonorgestrel-releasing intrauterine system –(Mirena®) PLUS male condom

6. TOXICITIES AND DOSE DELAYS/DOSE MODIFICATIONS

The NCI Common Terminology Criteria for Adverse Events (CTCAE) v5 will be used to grade adverse events.

Subjects enrolled in this study will be evaluated clinically and with standard laboratory tests before and at regular intervals during their participation in this study as specified in Study Calendar & Evaluations.

Subjects will be evaluated for adverse events (all grades), serious adverse events, and adverse events requiring study drug interruption or discontinuation as specified in Study Calendar & Evaluations.

6.1 Dose Delays/Dose Modifications

All toxicities that occur during the study will be actively managed following the standard of care unless otherwise specified in the protocol.

Patients who have the TAK-659 dose held due to treatment-related or possibly related AEs may resume study drug after resolution of the AE but may either maintain the same dose level or have doses of TAK-659 reduced (dose reduction) by at least 1 dose level. When a dose reduction occurs, the TAK-659 dose will be reduced to the next lower dose that either been established as a safe dose during dose escalation or is lower by a 20-mg increment**.** If initial dose adjustment does not provide sufficient relief, the dose of TAK- 659 can be further reduced if the treating physician considers that the patient is benefiting from study treatment and may benefit at a further reduced dose of TAK-659. When a dose reduction of TAK-659 is required due to toxicity, no dose re-escalation will be permitted.

If TAK-659 dosing is delayed for > 21 days for TAK-659–related or possibly related toxicities despite supportive treatment per standard clinical practice or more than 2 dose reductions are required in a patient, this patient should be considered to have study treatment discontinued, unless the treating physician considers that the patient may benefit from continued study treatment after resolution of AEs

to ≤ Grade 1, or baseline, or a level considered acceptable by the physician. The patient will continue to be followed for 30 days for safety evaluation after the last administration of TAK-659**.**

Patients who experience a DLT (during escalation) or DLT-like toxicity (during phase 2 of the study) during the first cycle will, in general, require that treatment with TAK-659 be permanently discontinued. If, in the opinion of the investigator it is in the patient's best interest to continue treatment with TAK-659, then the dose of TAK-659 will be reduced by at least 1 dose level when treatment resumes after recovery of the toxicity or toxicities in question to ≤ Grade 1 or to baseline values or to a level considered acceptable by the investigator. However, if a patient requires a dose delay of > 21 days for such an event to resolve despite the best supportive care permissible per protocol, then the patient must be discontinued from the study.

Detailed dose modification guidelines are provided in Section 6.2. In the case of TAK-659, which is administered once daily orally, a 1-step reduction of dose (by 20mg) may occur and if toxicity occurs at that dose, a second reduction may be considered. This second reduction will be subject to the judgment of the treating physician. In the case of Ixazomib, further reductions below the -2 dose step reduction from the starting dose that already exists below the RP2D in myeloma is not anticipated.

When a dose reduction occurs, no dose-reescalation will be permitted intrapatient. If study drug dosing is delayed for >21 days despite supportive treatment per standard clinical practice or more than 2 dose reductions are required in a patient, this patient will have study treatment discontinued.

There exist both unique and overlapping toxicities of TAK-659 and Ixazomib, which will influence dose modification of one or both within the combination. As compared with its predecessor Bortezomib, Ixazomib possesses lower incidence of neuropathy, but the incidence of diarrhea may be higher. However, that diarrhea is usually below grade 3, except in 15-20 % cases in the phase I weekly administration trial. Both Ixazomib and Bortezomib affect thrombocytopenia, but in the context of AML induction therapy, thrombocytopenia is an intrinsic feature, which is supported by transfusion.

By contrast, diarrhea occurs as AE with TAK-659, at 32% rate, and the grade is not high, but in preclinical animal models, gastrointestinal hemorrhage and/or bowel intimal damage was noted at high doses. In the phase Ib C34002 AML trial, the major DLTs included hemorrhage, possibly related to thrombocytopenia, or to functional inactivation of the platelets, at the highest dose of TAK-659: 80 mg po bid. In addition, asymptomatic elevation of lipase, AST and ALT have been observed frequently, but pancreatitis or hepatitis are rare. Pneumonitis felt related to TAK-659 is rare.

6.1.1 TAK-659/Ixazomib Dose Adjustments for Hematologic Toxicities

Blood counts should be regularly monitored during the study as specified in the Schedule of Events and tested more frequently if clinically indicated based on local standard practice. Comparison to baseline values and AE grading based on CTCAE (v.5) will be performed. When severe hematological AE's occur (≥ Grade 4 events, febrile neutropenic infection, thrombocytopenia with signs of overt bleeding or clinically significant bleeding), close monitoring and medical management using supportive care if indicated will be given based on standard practice (See Section 6.3) while the study drug should be continued. If these events persist despite supportive care (not resolved to ≤ Grade 1, or baseline, or a level considered acceptable by the investigator within 7 days), at the investigator's discretion, bone marrow aspirate assessment will be performed to evaluate whether the prolonged myelosuppression is due to leukemic infiltration or disease progression. If leukemic blasts are ≤ 5% in bone marrow and

bone marrow aplasia is seen, the study drugs should be continued for completion of the current cycle if less than 14 days, with repeat bone marrow aspirate in the absence of peripheral hematologic recovery to assess marrow cellularity. In the absence of evidence for bone marrow recovery, the study drugs should be withheld for up to 21 days. Clinical and laboratory re-evaluation should be repeated at least weekly or more frequently until recovery to ≤ Grade 1 or baseline or a level considered acceptable by the investigator. Upon recovery, TAK-659 may be reinitiated (along with Ixazomib) at the same dose level or at a reduced dose level at the discretion of the investigator. If no recovery occurs within 21 days (based on peripheral blood counts and/or repeat bone marrow aspirate) after the study drug hold (42 days in total after the first dose on Cycle 1, Day 1), the patient should discontinue study treatment, and if this occurs in Cycle 1, the patient will be considered to have a DLT. The patient will continue to be followed until resolution or stabilization of the event, initiation of another anti-leukemia therapy, or overt AML progression, whichever occurs first.

When a dose reduction of TAK-659 or ixazomib is required in a patient, no re-escalation of dose will be permitted.

6.1.2 TAK-659 dose modifications for Nonhematologic Toxicities

Please refer to the Table below for dose held and dose reduction recommendations for non-hematologic toxicities. When the dose of TAK-659 is withheld based on the following criteria, clinical and laboratory re-evaluation should be repeated at least weekly or more frequently until the toxicity resolves to ≤Grade 1, or baseline, or a level considered acceptable by the treating investigator (must be ≤Grade 2). Upon recovery, TAK-659 may be reinitiated either at the same dose level or at a reduced dose level. For events in which there are transient lab value abnormalities that, based on investigator assessment, are not clinically significant or related to disease and not the drug, continuation of therapy without following the dose modification guideline is permissible after discussion with the sponsor investigator.

Criteria	Action
All Grade 3 Nonhematologic Toxicities with the exception of: • Grade 3 nausea, vomiting and diarrhea resolved to ≤ Grade 1 or baseline within 48 hours with optimal antiemetics and antidiarrheal following SOC • Transient Grade 3 fatigue (lasting < 72 hours) • Asymptomatic lipase elevation (< Grade 4) in the absence of significant amylase elevation (< Grade 3) considered not dose limiting • Asymptomatic amylase elevation (< Grade 4) in the absence of lipase elevation (< Grade 3) considered not dose limiting • Asymptomatic Grade 3 elevation of a single liver enzyme (AST or ALT) in the absence of significant bilirubin elevation (< Grade 3) considered not dose limiting • Grade 3 hypophosphatemia resolved to Grade ≤ 1 or baseline within 72 hours with phosphate repletion • Other asymptomatic Grade 3 laboratory abnormalities that the investigator considers not clinically significant	• Hold TAK-659 until resolution to Grade ≤ 1 or baseline or a level considered acceptable by the investigator (must be ≤Grade 2). o If resolved in ≤ 7 days, then maintain the dose level. o If resolved in > 7 days, then dose reduce by 1 dose level. o If recurs, then dose reduce by 1 dose level. • For the exceptions listed, maintain dose level (no dose hold required). • Permanent discontinuation should be considered if the toxicities persist as ≥Grade 3 for more than 21 days despite temporary disruption of study drug.
Grade 4 Nonhematologic Toxicities	• Permanently discontinue TAK-659 for any grade 4 nonhematologic toxicities that are at least possibly related to TAK-659.

In general, the study drug(s) will resume only after the resolution of AEs to ≤Grade 1 or baseline or as specified in these dose modification guidelines. However, retreatment with study drug(s) could start when the AEs are resolved to a level deemed acceptable by the investigator. When a dose reduction of TAK-659 is required, no re-escalation of dose will be permitted.

6.1.3 Ixazomib Dose Adjustments for Hematologic Toxicities

Ixazomib Dose Adjustments for Hematologic Toxicities	
Criteria	**Action**
Within-Cycle Dose Modifications **In Induction (< 2 cycles and marrow blasts present)**	• Dose reduction is not required
Within-Cycle Dose Modifications **In consolidation (after documentation of morphologic blast clearance, generally beginning after the second cycle)**	• See below
• If platelet count ≤ 20×10^9/L or ANC ≤ 0.20×10^9/L on a ixazomib dosing day (other than Day 1)	• Ixazomib dose should be withheld. • Complete blood count (CBC) with differential should be repeated at least twice weekly until the ANC and/or platelet counts have exceeded the prespecified values. • Upon recovery, ixazomib may be reinitiated with 1 dose level reduction.
Dose Modifications for Subsequent Treatment Cycles	
• Delay of 1 week in the start of a subsequent cycle due to lack of toxicity recovery as ANC < 0.5×10^9/L, platelet count < 30×10^9/L	• Hold ixazomib until resolution as per criteria • Upon recovery, reduce ixazomib 1 dose level. • The maximum delay before treatment should be discontinued will be 2 weeks or at the discretion of the PI.
Dose Modifications for Subsequent Treatment Cycles	
• All hematologic toxicities	• For hematologic toxicity that occurs during a cycle but recovers in time for the start of the next cycle,: o If dose was reduced within the cycle, start the next cycle at that same dose. o If due to toxicity timing, ie, after Day 15 dosing thus a dose reduction was not required at that point in the cycle, reduce ixazomib by 1 dose level at the start of that cycle. o Do not reduce the dose both within a cycle and at the start of the cycle for the same most severe toxicity.

Ixazomib Treatment Modification (Delays, Reductions, and Discontinuations) Due to Adverse Events (Non-Hematologic Toxicities)		
Adverse Event (Severity)	**Action on Study Drug**	**Further Considerations**
Peripheral Neuropathy: Grade 1 peripheral neuropathy	• No action	Grade 1 signs and symptoms: asymptomatic; without pain or loss of function; clinical or diagnostic observations only [27]
New or worsening Grade 1 peripheral neuropathy with pain or Grade 2	• Hold study drug until resolution to Grade ≤ 1 or baseline	Grade 2 signs and symptoms: Moderate symptoms; limiting instrumental activities of daily living (ADL) [27]
New or worsening Grade 2 peripheral neuropathy with pain or Grade 3	• Hold study drug until resolution to Grade ≤ 1 or baseline • Reduce study drug to next lower dose upon recovery	Grade 3 signs and symptoms: severe symptoms; limiting self-care ADL; assistive device indicated [27]
New or worsening Grade 4 peripheral neuropathy	• Discontinue study drug	
Grade 2 Rash	• Symptomatic recommendations as per section 6.3	The investigator and project clinician may discuss considerations for dose modifications and symptom management.
Grade 3 nonhematologic toxicity judged to be related to study drug	• Hold study drug until resolution to Grade < 1 or baseline	Symptomatic recommendations noted in Section 6.3
If not recovered to < Grade 1 or baseline within 4 weeks	• Reduce study drug 1 to next lower dose upon return to < Grade 1 or baseline	
Subsequent recurrence Grade 3 that does not recover to < Grade 1 or baseline within 4 weeks	• Hold study drug until resolution to Grade < 1 or baseline • Reduce study drug to next lower dose	Monitor closely, take appropriate medical precautions, and provide appropriate symptomatic care
Grade 4 nonhematologic toxicities judged to be related to study drug	• Consider permanently discontinuing study drug	Exceptions are cases in which the investigator determines the patient is obtaining a clinical benefit

Once ixazomib is reduced for any toxicity, the dose may not be re-escalated.

6.2 Dose Levels for Dose Reduction

Dose level	**Dose of TAK-659** Days 1-15	**Dose of Ixazomib** Days 1, 8, 15		
Starting Dose	100mg po once daily	2.3 mg	3mg	4mg
Dose level (-1)	80mg po once daily	Contact sponsor-investigator for consideration of schedule below 2.3mg.	2.3mg	3mg
Dose level (-2)	60mg po once daily	--	Contact sponsor-investigator for consideration of schedule below 2.3mg.	2.3mg.
If study drug dosing is delayed for >21 days despite supportive treatment per standard clinical practice or more than 2 dose reductions are required in a patient, this patient will have study treatment discontinued.				

6.3 Management of Clinical Events

Therapies that are required to manage AEs and control cancer symptoms are allowed based on standard clinical practice, unless specifically excluded. Supportive care agents, such as erythropoietin, G-CSF, blood products (RBC and platelet transfusions), and pain medications are permitted as needed per American Society of Hematology (ASH)/ASCO guidelines or local institutional practice. However, these agents should not be used in this study in a manner that would either help establish eligibility for the study or support escalation of study drug dose during dose escalation.

6.3.1 Prophylaxis Against Infection

Patients with advanced hematological malignancies may be at an increased risk of infection. Prophylactic use of antibiotic, antiviral, or antifungal medication can be considered as clinically indicated and as per local standard practice. Ciprofloxacin is considered a standard for patients with ANC <500. Antifungal prophylaxis with fluconazole or isovuconazole which are a moderate CYP inhibitory agents is permitted with dose reduction of TAK-659 as in 5.3.1. Patients requiring prophylaxis with fluconazole or patients whom develop active fungal infection, continuation of TAK-659 in the combination will require dose reduction to 60mg po daily of the TAK-659 with the mold-active agent isovuconazole, and careful analysis of toxicity parameters, especially including QTc and hepatic and pancreatic enzymes. Dosing of Ixazomib will not require modification in the absence of relatable toxicity. The above determined TAK-659 dose cannot be escalated. If toxicity supervenes, the patient will be taken off study for alternative anti-infective therapy as needed. Should no toxicity be observed in a cohort, stepwise dose escalation, first to 80mg, may be considered.

Antiviral therapy such as acyclovir may be initiated as clinically indicated.

The severe and prolonged period of neutropenia seen with therapy is frequently associated with neutropenic fevers and a high risk of infection with bacteria or fungi and viral reactivation. To minimize

the risk of infection, it is recommended that patients be placed on "neutropenic precaution", with or without the addition of prophylactic antibiotics, antifungals, or antivirals. In addition, patients should be screened for possible infectious foci (eg, dental status).

The components of "neutropenic precaution" while inpatient may vary by institution but most commonly include: a high efficiency particulate air-filtered room, a diet free of raw berries or vegetables grown in dirt, no sick visitors, and no smoking. In addition, hand washing by all visitors and caregivers should be strictly enforced.

Consideration should be given to antibiotic, antifungal, and antiviral prophylaxis during therapy, either as an outpatient or as an inpatient; however, the use of such agents should be at the discretion of the treating physician based on the local standard practice. Patients who develop neutropenic fever should be evaluated promptly and treated immediately with parental antibiotics tailored to the prominent organisms and resistance patterns of the institution.

Patients with lymphopenia and neutropenia may be more prone to developing infections, such as respiratory tract infections or pneumonia. Consider a diagnosis of opportunistic infection including *Pneumocystis jirovecii* pneumonia (PJP, formerly known as *Pneumocystis carinii* pneumonia) in patients presenting with shortness of breath, cough, or fever. Prophylaxis for PJP may be initiated (either at baseline or during treatment) if clinically indicated. For older patients, patients with recent exposure to steroids or immunosuppressive agents, or patients who, in the investigator's opinion, are more susceptible to opportunistic infection at baseline, PJP prophylaxis should be considered at the start of the study treatment. When steroids and/or any immunomodulatory agents need to be used to manage the AEs during the study, PJP prophylaxis should be considered when the study treatment resumes or is coadministered. Trimethoprim-sulfamethoxazole is recommended as the treatment of choice for PJP prophylaxis unless contraindicated. However, investigator discretion in selecting a more appropriate prophylaxis regimen for their patients is permitted.

Myelosuppression can also be associated with reactivation of herpes zoster, cytomegalovirus (CMV), herpes simplex and other viruses. Antiviral therapy such as acyclovir, gancyclovir, valacyclovir, or other antiviral agents may be initiated as clinically indicated. Testing of CMV replication by a local polymerase chain reaction (PCR) assay will be required at baseline, and further monitoring and prophylactic or preemptive therapy to asymptomatic patients, if indicated, should follow the institutional standard practice. The following agents should be considered for prophylaxis or preemptive treatment against CMV: ganciclovir intravenously (IV), valganciclovir (orally), foscarnet (IV), or cidofovir (IV). Duration of antiviral therapy generally is for at least 2 weeks until CMV is no longer detected by PCR. Institutional guidelines may be used in the management of viral reactivation.

6.3.2 Leukocytosis

For patients who develop symptoms of leukocytosis (WBC > 100,000/mm^3) while on the study, TAK-659 treatment should be withheld until the leukocytosis symptoms are controlled. Treatment of leukocytosis symptoms may include leukapheresis and hydroxyurea administration (concurrent use of hydroxyurea with the study drug is only permitted during the first 21 days of study treatment) per institutional guidelines. When the WBC of the patient is < 50,000/mm^3 and symptoms are improved, TAK-659 treatment may be restarted after consulting with the project clinician.

6.3.3 Prophylaxis Against Risk of Reactivation of Herpes Infection

Patients may be at an increased risk of infection including reactivation of herpes zoster and herpes simplex viruses. Antiviral therapy such as acyclovir, valacyclovir, or other antivirals may be initiated as clinically indicated. Other antivirals are also acceptable.

6.3.4 Pneumonitis

Patients with serious lung events that do not respond to conventional antimicrobial therapy should be assessed for drug-induced pneumonitis after ruling out infectious causes and alternative etiologies. If pneumonitis is suspected, TAK-659/Ixazomib treatment should be interrupted and the patient treated per standard of care. If pneumonitis is moderate/severe, discontinue TAK-659/Ixazomib. Patients should be monitored for respiratory signs and symptoms throughout treatment and be advised to promptly report respiratory symptoms.

6.3.5 Nausea and/or Vomiting

Prophylactic antiemetics before the first dose of the study drug is not recommended. Antiemetics may be used prophylactically as clinically indicated following the occurrence of a first event of TAK-659/Ixazomib drug-related or possibly related nausea and/or vomiting.

6.3.6 Diarrhea

Prophylactic antidiarrheals is not recommended before the first dose of study drugs. However, diarrhea should be managed according to clinical practice, including the administration of antidiarrheals once infectious causes are excluded. Fluid intake should be maintained to avoid dehydration. Fluid deficit should be corrected before initiation of treatment and during treatment.

6.3.7 Edema (Including Periorbital)

Peripheral and periorbital oedema have been observed in patients treated with TAK-659. Management of the event should follow the standard local practice and dose modification as required.

6.3.8 Rash With or Without Prutitus

Prophylactic measures should also be considered if a patient develops a rash (eg, using a thick, alcohol-free emollient cream on dry areas of the body). In the case of rash, the use of a topical or oral steroid (eg, prednisone ≤ 10 mg per day or equivalent) is permitted.

In line with clinical practice, dermatology consult and biopsy of Grade 3 or higher rash or any SAE involving rash is recommended. Prophylactic measures should also be considered if a patient has previously developed a rash (eg, using a thick, alcohol-free emollient cream on dry areas of the body or oral or topical antihistamines). A rare risk is Stevens-Johnson Syndrome, a severe and potentially life-threatening rash with skin peeling and mouth sores, which should be managed symptomatically according to standard medical practice. Punch biopsies for histopathological analysis are encouraged at the discretion of the investigator.

6.3.9 Thrombocytopenia

Blood counts should be monitored regularly. Platelet transfusion is allowed to manage severe thrombocytopenia to prevent and minimize bleeding according to ASH/ASCO guidelines and should be proactive to consider functional defects within platelets as a result of Syk/Tec inhibition. Every attempt should be made to adjust the platelet count threshold to a higher level (beginning at 10k without

demonstrated bleeding) at any signs of bleeding, such as oral purpura. Each transfusion episode, including the type of transfusion (platelets), should be recorded.

Ixazomib administration should be modified as noted as per dose modification recommendations in the protocol when thrombocytopenia occurs, after remission has been established by bone marrow testing. In that context, continuation therapy can be reinitiated at a reduced level upon recovery of platelet counts. However, prior to attainment of remission or in the period prior to completion of cycle 2, Ixazomib administration should not be altered due to thrombocytopenia alone, if it can be ameliorated by transfusion to attain minimum platelet count 10,000/uL.

A rare risk is thrombotic thrombocytopenic purpura (TTP), a rare blood disorder where blood clots form in small blood vessels throughout the body characterized by thrombocytopenia and hemolysis, petechiae, fever, or possibly more serious signs and symptoms. TTP should be managed symptomatically according to standard medical practice.

6.3.10 Neutropenia

Blood counts should be monitored regularly. Myeloid growth factors (eg, G-CSF, GM-CSF) may be used to treat severe and/or febrile neutropenia according to ASCO guidelines. However, it should be noted that growth factors are not used routinely in AML patients undergoing chemotherapy and prophylactic use of myeloid growth factors should be avoided during the first cycle of dose escalation.

6.3.11 Anemia

Hemoglobin should be monitored regularly. Packed RBC transfusion is permitted, as necessary, per local institutional practice. In general, RBC transfusion is recommended for all symptomatic patients with anemia or any asymptomatic patients with a hemoglobin $\leq$ 7 to 8 g/dL with the purpose of maintaining the hemoglobin between 8 and 10 g/dL depending on patients' age, symptoms, and comorbid conditions. Each transfusion episode, including the type of transfusion (RBC), should be recorded. Erythropoietic agent use at the investigator's discretion is also allowed and should be administered according to institutional practice.

6.3.12 Hypophosphatemia

Hypophosphatemia has been observed in patients treated with TAK-659. Consider prophylaxis; otherwise refer to dose modification guideline.

6.3.13 Enzyme Elevations (Transaminase, Amylase and Lipase, CPK and LDH Elevations)

Elevations of the enzymes above have been observed. Events are generally asymptomatic and reversible with dose interruption.

LDH elevations have been observed in the majority of patients exposed to TAK-659. These elevations have been asymptomatic and the clinical significance is unknown. No doses have been interrupted due to increased LDH; however, LDH elevations have been observed to be reversible in patients who had TAK-659 interrupted due to other reasons.

6.3.14 Fluid Deficit

Fluid deficit should be corrected before initiation of study drug and during treatment.

6.3.15 Hypotension

Symptomatic hypotension and orthostatic hypotension with or without syncope have been reported with ixazomib. Blood pressure should be closely monitored while the patient is on study treatment and fluid deficit should be corrected as needed, especially in the setting of concomitant symptoms such as nausea, vomiting, diarrhea, or anorexia. Patients taking medications and/or diuretics to manage their blood pressure (for either hypo- or hypertension) should be managed according to standard clinical practice, including considerations for dose adjustments of their concomitant medications during the course of the trial. Fluid deficit should be corrected before initiation of study drug and as needed during treatment to avoid dehydration.

6.3.16 Posterior Reversible Encephalopathy Syndrome

One case of posterior reversible encephalopathy syndrome, which ultimately resolved, has been reported with ixazomib. This condition is characterized by headache, seizures and visual loss, as well as abrupt increase in blood pressure. Diagnosis may be confirmed by magnetic resonance imaging (MRI). If the syndrome is diagnosed or suspected, symptom-directed treatment should be maintained until the condition is reversed by control of hypertension or other instigating factors.

6.3.17 Transverse Myelitis

Transverse myelitis has also been reported with ixazomib. It is not known if ixazomib causes transverse myelitis; however, because it happened to a patient receiving ixazomib, the possibility that ixazomib may have contributed to transverse myelitis cannot be excluded.

6.4 Protocol Therapy Discontinuation

Patients will be informed that they have the right to withdraw from the study at any time for any reason, without prejudice to their medical care. In addition to discontinuation from therapy related to toxicities as outlined in section 6.1, a subject will also be discontinued from protocol therapy and followed up per protocol under the circumstances outlined below. The reason for discontinuation of protocol therapy will be documented on the electronic case report form (eCRF).

- Documented disease progression
- The treating physician determines a change of therapy would be in the best interest of the subject
- The subject requests to discontinue protocol therapy, whether due to unacceptable toxicity or for other reasons
    - If a subject decides to prematurely discontinue protocol therapy (“refuses treatment”), the subject should be asked if he or she may still be contacted for further scheduled study assessments. The outcome of that discussion should be documented in both the medical records and in the eCRF.
- A female subject becomes pregnant
- If protocol therapy is interrupted for ≥ 21 days.
- Protocol violation or non-compliance of the patient
- Study terminated
- If the responsible physician thinks a change of therapy would be best for the patient
- Noncompliance of the patient
- Pregnancy
- Death

6.5 Completion of Study

Patients will be considered to have completed the study if:

- They are followed until death before the end of the survival follow-up window (up to 12 months after discontinuation of study drug for any reason)
- They remain on study treatment free of disease progression at the close of the study, at least 1 year after their first dose of study treatment.
- They continue on to the follow-up for survival after discontinuation of the study drug and reach the end of the 12-month OS follow-up window.
- They discontinue study treatment while in CR and continue on to the follow-up for progression and either experience disease progression before the end of the 12-month follow-up period or reach the end of the follow-up period
- The sponsor investigator or funder (Millennium Takeda) terminates the study

6.6 Protocol Discontinuation

If a subject decides to withdraw from the study (and not just from protocol therapy) all efforts should be made to complete the final study assessments. The site study team should contact the subject by telephone or through a clinic visit to determine the reason for the study withdrawal. If the reason for withdrawal is an adverse event, it will be recorded on the eCRF.

7. STUDY CALENDAR: DOSE ESCALATION COHORTS (PHASE 1)

	Screen	Cycle 1				Cycles 2-4			Cycles 5+			Safety follow up	Long-term follow up
Cycle = 21days	-28 days[1]	D 1	D 4	D 8	D 15	D 1	D 8	D 15	D 1	D 8	D 15	30 days post Tx[2]	Q month (±14)[3]
REQUIRED ASSESSMENTS													
Informed consent[4], Eligibility criteria	X												
Medical history, Disease characteristics	X	X											
Physical exam[5]	X	X[5]		Sx	Sx	X		Sx	X			X	
Ophthalmic Exam[6]	X					C2			C7D1; q6			X	
Height (screen only); Weight	X	X				X			X			X	
Vital signs (BP, pulse, temperature)	X	X		X	X	X		X	X			X	
ECOG Performance status	X	X				X			X			X	
12-lead ECG[7]	X	×2			×2			X	X			X	
AEs & concomitant medications[8]		X		X	X	X		X	X			X	
Drug Diary Review		X		X	X	X			X			X	
LABORATORY ASSESSMENTS[9]													
CBC with diff, platelets, ANC. Chemistry[10]	X	X[10]	X	X[10]	X	X	C2	X	X			X	
Uric acid, Mag, Amylase, Lipase, GGT[10]	X	X[10]	X	X[10]	X	X		X	X			X	
Urinalysis	X				X	X		X	X			X	
Pregnancy test for WOCBP[11]	X	X				X			X				
DISEASE ASSESSMENT													
NGS results or submit for NGS testing	X												
Bone marrow biopsy and aspirate[12]	X				X			C2,4	as indicated				
TREATMENT EXPOSURE													
TAK-659 (Daily ×15 days)		D1-15				D1-15			D1-15				
Ixazomib (Days 1, 8, 15)		X		X	X	X	X	X	X	X	X		
CORRELATIVE STUDIES (SPECIMEN COLLECTION)													
Buccal swab for germline DNA analysis		X											
Bone marrow aspirate for biomarker analyses and PD	X		X[13]		X			C2,4					
Serum for proteomic analysis		X	X										
Plasma for PK[14]		×2			×2			×2					
BANKING SAMPLES (SPECIMEN COLLECTION)													
Whole Blood[15]		X											
Serum and Plasma[15]		X										X	
FOLLOW-UP													
Survival status, subsequent therapy													X

Phase I calendar footnotes:

Evaluations/laboratory assessments performed on visit days will occur before dosing unless otherwise indicated. On these days, patients will be instructed to hold dosing until relevant assessments have been completed.

1. Screening assessments performed within 3 days before Cycle 1 Day 1 do not need to be repeated, unless otherwise specified.
2. Safety follow up visit will occur 30 days (+10 days) after the last dose of study drug or before the start of subsequent anticancer therapy (other than hydroxyurea) whichever occurs first.
3. All patients, including those no longer on treatment, will be followed for survival. Patients who discontinue the study will be followed for survival every month for up to 12 months after discontinuation of the study drug until death, loss to follow-up, or withdrawal of consent. Information on subsequent anticancer therapies will also be collected. For patients who achieve CR but discontinue study treatment while still in remission, disease progression based upon available local data will also be collected.
4. Informed consent may be obtained before the Screening period (28 days before Cycle 1 Day 1 dosing).
5. Complete physical examination (PE) will be done at screening, including a neurological exam. Complete PEs will also be done on Day 1 of each cycle and at the 30-day Safety follow up visit. Symptom- or finding-directed PEs will be done on C1D8 and 15 and C2-4 D15.
6. An ophthalmic exam will be done at screening, C2D1, C7D1, q 6 cycles thereafter (±2 wks), and at 30-day Safety follow up visit. See Section 8.1.
7. Single 12-lead ECGs will be performed within 1 hour predose and 3 hours postdose (±15 min) on C1D1 and C1D15. All other ECGs will be done only at predose. When the timing of a blood sample coincides with the timing of an ECG, the ECG will be completed first.
8. AEs & con meds will be recorded from first dose of study drugs through 30 days after the last dose of study drugs or the start of subsequent anticancer therapy, whichever occurs first. **SAEs** will be reported from signing of informed consent through 30 days after the last dose of study drug, even if the patient starts nonprotocol therapy.
9. Laboratory assessments may be conducted within -3 days of the scheduled visit, except PK/PD assessments, or unless otherwise noted. Day 1 visits of Cycle 2 and beyond may be modified by up to 3 days due to inclement weather, holidays, vacations, etc.
10. The hematology and chemistry blood samples for C1D1 may be collected within 3 days before dosing to ensure patient eligibility on Day 1. The percentage of leukemic blast cells should be noted in the hematology panel. Monitor CBCs and CMPs at least twice weekly for the first 2 weeks (including tumor lysis labs) and then check CBCs and CMPs at least weekly to complete the first 2 cycles.
11. Women of childbearing potential: A serum pregnancy test will be done at screening. A urine pregnancy test will be done predose D1 of all cycles, with negative results available prior to dosing. If a negative serum test is done within 3 days of C1D1, the C1D1 urine test will be waived.
12. Bone marrow biopsy and aspirate for disease burden (blast counts), cytogenetics, and cellular composition by flow cytometry will be done at screening. In addition, BM biopsies and/or aspirates will be done to assess disease response at Cycles 1, 2, and 4 between Days 15-21, provided the results are available before Day 1 of the next cycle. After Cycle 4, BM biopsy and/or aspirate assessment will be performed as clinically indicated based on changes in peripheral blood counts, or when it is needed to establish either CR or disease progression. NOTE: a BM biopsy is required only at screening. Residual and/or additional bone marrow samples will be requested for biomarker analysis. In cases when insufficient bone marrow aspirate is collected, peripheral blood will be collected and submitted per the instructions in the CLM.
13. Bone marrow will be done at C1D4 for the 1st 5 patients. Thereafter, BM timing (D4 vs 8) will determined from the results in the first 5 patients.
14. Blood for PK plasma samples will be collected at: C1D1 (pre-dose, 2-4 hr post-dose), C1D15 (pre-dose, 2-4 hr post-dose), Cycle 2, 3, 4 Day 15 (pre-dose, post-dose anytime during clinic visit). Instruct patient to withhold study drug(s) for that day. The timing of the visit should be planned so that the pre-dose PK draw occurs near the same time as the previous day's dosing. When a blood sample coincides with the timing of an ECG, the ECG will be completed first. Refer to the CLM for detailed instructions on collection, processing, storing, and shipping of PK samples.
15. Whole blood for banking is to be collected at pre-dose C1D1. Serum and plasma for banking are to be collected at pre-dose C1D1 and at the 30-Day Safety Follow up visit. See CLM for collection, processing, labeling and shipping instructions.

8. STUDY CALENDAR: PHASE 2

	Screen	Cycle 1				Cycles 2-4			C 3	Cycles 5+			Safety follow up	Long-term follow up
Cycle = 21days	-28 days[1]	D 1	D 4	D 8	D 15	D 1	D 8	D 15	D 22	D 1	D 8	D 15	30 days post Tx[2]	Q month (±14)[3]
REQUIRED ASSESSMENTS														
Informed consent[4], Eligibility criteria	X													
Medical history, Disease characteristics	X	X												
Physical exam[5]	X	X[5]		Sx	Sx	X		Sx		X			X	
Ophthalmic Exam[6]	X					C2				C7D1; q6			X	
Height (screen only); Weight	X	X				X				X			X	
Vital signs (BP, pulse, temperature)	X	X		X	X	X		X		X			X	
ECOG Performance status	X	X				X				X			X	
12-lead ECG[7]	X	X						X		X			X	
AEs & concomitant medications[8]		X			X	X		X		X			X	
Drug Diary Review		X			X	X				X			X	
LABORATORY ASSESSMENTS[9]														
CBC with diff, platelets, ANC. Chemistry[10]	X	X[10]	X	X[10]	X	X	C2	X		X			X	
Uric acid, Mag, Amylase, Lipase, GGT[10]	X	X[10]	X	X[10]	X	X		X		X			X	
Urinalysis	X			X		X		X		X			X	
Pregnancy test for WOCBP[11]	X	X				X				X				
DISEASE ASSESSMENT														
NGS results or submit for NGS testing	X													
Bone marrow biopsy and aspirate[12]	X				X				C3D22[12]	as indicated				
TREATMENT EXPOSURE														
TAK-659 (Daily ×15 days)		D1-15				D1-15				D1-15				
Ixazomib (Days 1, 8, 15)		X		X	X	X	X	X		X	X	X		
CORRELATIVE STUDIES (SPECIMEN COLLECTION)														
Buccal swab for germline DNA analysis		X												
Bone marrow aspirate for biomarker analyses and PD	X		X[13]		X				C3D22[12]	@ PD[13]				
Serum for proteomic analysis		X	X											
Plasma for PK[14]		×2			×2			×2						
BANKING SAMPLES (SPECIMEN COLLECTION)														
Whole Blood[15]		X												
Serum and Plasma[15]		X											X	
FOLLOW-UP														
Survival status, subsequent therapy														X

Phase II calendar footnotes: Evaluations/laboratory assessments performed on visit days need to take place before dosing unless otherwise indicated. On these days, patients should be instructed to hold dosing until relevant assessments have been completed.

1. Screening assessments performed within 3 days before Cycle 1 Day 1 do not need to be repeated, unless otherwise specified.
2. Safety follow up visit will occur 30 days (+10 days) after the last dose of study drug or before the start of subsequent anticancer therapy (other than hydroxyurea) whichever occurs first.
3. All patients, including those no longer on treatment, will be followed for survival. Patients who discontinue the study will be followed for survival every month for up to 12 months after discontinuation of the study drug until death, loss to follow-up, or withdrawal of consent. Information on subsequent anticancer therapies will also be collected. For patients who achieve CR but discontinue study treatment while still in remission, disease progression based upon available local data will also be collected.
4. Informed consent may be obtained before the Screening period (28 days before Cycle 1 Day 1 dosing).
5. Complete physical examination (PE) will be done at screening, including a neurological exam. Complete PEs will also be done on Day 1 of each cycle, and at the 30-day Safety follow up visit. Symptom- or finding- directed PEs will be done on C1D8 and 15 and C2-4 D15.
6. An ophthalmic exam will be done at screening, C2D1, C7D1, q 6 cycles thereafter (±2 wks), and at 30-day Safety follow up visit. See Sect. 8.1.
7. Single 12-lead ECGs will be done at screening, predose during each cycle and at the 30-day Safety follow up visit. When the timing of a blood sample coincides with the timing of an ECG, the ECG will be completed first.
8. AEs & con meds will be recorded from first dose of study drugs through 30 days after the last dose of study drugs or to the start of subsequent anticancer therapy, whichever occurs first. SAEs will be reported from signing of the informed consent form through 30 days after the last dose of study drug even if the patient starts nonprotocol therapy.
9. Laboratory assessments may be conducted within - 3 days of the scheduled visit, except PK/PD assessments, or unless otherwise noted. Day 1 visits of Cycle 2 and beyond may be modified by up to 3 days due to inclement weather, holidays, vacations, etc.
10. The hematology and chemistry blood samples for C1D1 may be collected within 3 days before dosing to ensure patient eligibility on Day 1. The percentage of leukemic blast cells should also be noted in the hematology panel. Monitor CBCs and CMPs at least twice weekly for the first 2 weeks (including tumor lysis labs) and then check CBCs and CMPs at least weekly to complete the first 2 cycles.
11. Women of childbearing potential: A serum pregnancy test will be performed for at screening. A urine pregnancy test will be done predose D1 of all cycles, with negative results available prior to dosing. If a negative serum test is done within 3 days of C1D1, the C1D1 urine test is waived.
12. Bone marrow biopsy and aspirate for disease burden (blast counts), cytogenetics, and cellular composition by flow cytometry will be done at screening. In addition, BM aspirates and/or biopsies will be done to assess disease response at Cycle 1 Day 15 and Cycle 3 Day 22, so that the results are available before Day 1 of the next cycle. In the post-Cycle 3 assessment, aspirate and biopsy are suggested on day 22 (or within 3 days prior to C4D1), simultaneous with the plan to continue onto C4 D1, to serve as record establishing the optimal response/composite CR. Such planning should be guided by the trend of blast reduction observed during Cycles 1, 2. After Cycle 3 assessment, BM biopsy and/or aspirate assessment will be performed as clinically indicated based on changes in peripheral blood counts, or when it is needed to establish either CR or disease progression. NOTE: a BM biopsy is required at screening and post-Cycle 3 D22. Residual and/or additional bone marrow samples will be requested for biomarker analysis. In cases when insufficient BM aspirate is collected, peripheral blood will be submitted per the instructions in the CLM.
13. BM timing (D4 vs D8) will be determined from results in the Phase I patients. At the time of relapse (PD) of any patient who had initially responded to TAK-659, bone marrow aspirate and/or biopsy materials will be collected for biomarker study.
14. Blood for PK plasma samples will be collected at : C1D1 (pre-dose, 2-4 hr post-dose), C1D15 (pre-dose, 2-4 hr post-dose), Cycle 2, 3, 4 Day 15 (pre-dose, post-dose anytime during clinic visit). Instruct patient withhold study drug(s) for that day. The timing of the visit should be planned so that the pre-dose PK draw occurs near the same time as the previous day's dosing. When a blood sample coincides with the timing of an ECG,

the ECG will be completed first. Refer to the CLM for detailed instructions on collection, processing, storing, and shipping of PK samples.

15. Whole blood for banking is to be collected at predose C1D1. Serum and plasma for banking are to be collected at pre-dose C1D1 and at the 30-Day Safety Follow up visit. See CLM for collection, processing, labeling and shipping instructions.

8.1 Study Procedures

Patients will be evaluated at scheduled visits over the following study periods: Screening, Treatment, and Safety Follow up. Evaluations during the Screening period are to be conducted within 28 days before administration of the first dose of study drug. Procedures conducted during the Screening period that are performed within 3 days of Cycle 1, Day 1 may also be used as the predose evaluation and do not need to be repeated, unless otherwise specified.

The timing of PK, PD, and proteomic assay assessments is specified in the Schedule of Events and is not flexible. Other laboratory assessments and procedures may occur within -3 days before the scheduled day, and Day 1 of Cycle 2 and beyond may be modified by up to 3 days due to extenuating circumstances (ie, inclement weather, holidays, vacations, or other administrative reasons).

Medical History
During the Screening period, a complete medical history will be compiled for each patient. The history will emphasize the background and progress of the patient's malignancy and include a description of prior therapies received. Sites will be required to record any screening reports on tumor cytogenetics and/or mutation assays (eg, FLT-3, and NGS) performed as part of the standard of care.

Physical Examination
A physical examination (PE) will be completed per standard of care at the times specified in the Schedule of Events. During dose escalation, complete PEs will be performed at screening, Day 1 of each cycle of treatment, and safety follow up. Symptom- or finding-directed PEs will be performed on Day 8 and 15 of Cycle 1 and Day 15 of Cycles 2, 3, and 4.

Note: Physical examinations at screening will include a neurological examination.

Height and Weight
Height will be measured only during screening (within 28 days before the first dose of TAK-659). Weight will be measured during the times specified in the Schedule of Events.

Vital Signs
Vital sign measurements include diastolic and systolic blood pressure, heart rate, and temperature, and will be assessed as specified in the Schedule of Events. Blood pressure should be determined with the patient in a seated position after the patient has been sitting quietly for approximately 5 minutes.

Pregnancy Test
A serum pregnancy test will be performed for women of childbearing potential at screening. A urine pregnancy test will be performed predose on Day 1 of all cycles with negative results available before the first dose may be administered. If the serum pregnancy test is performed within 3 days from the first dose and the result is negative, the urine pregnancy test on Cycle 1, Day 1 may be waived.

Concomitant Medications and Procedures
Concomitant medications and procedures will be recorded in the eCRF from the time of the first dose of study drug through 30 days after the last dose of study drug or to the start of subsequent anticancer

therapy, whichever occurs first. See Section 5.8 for a list of medications and therapies that are allowed/ prohibited during the study.

Electrocardiogram
12-lead ECGs will be performed and interpreted locally. The ECG schedule is more intensive for patients enrolled in the dose escalation cohorts. The time points for ECG collection may be revised based on emerging PK data, but the number of time points will not increase.

All scheduled ECGs should be performed pre-dose, unless otherwise specified, and after the patient has rested quietly for at least 5 minutes in a supine position. When the timing of a PK, PD, PIA or safety laboratory blood sample coincides with the timing of ECG measurements, the ECG will be completed before the collection of the blood sample. In some cases, it may be appropriate to repeat an abnormal ECG to rule out improper lead placement as contributing to the ECG abnormality.

Estimates of QTc for study eligibility should use QT correction formula (QTcF). In the event that a QTc value confirmed by the qualified reader is>475 msec, an evaluation to determine etiology should be conducted. If the prolonged QTc finding can be corrected based on change in medication and/or corrections of electrolyte abnormalities, and a repeat ECG meets eligibility requirements, the patient may enroll to the study upon review and agreement by the sponsor investigator.

Following initiation of treatment, if a QTc value is confirmed as>500 msec for any ECG, the following will occur:
- The sponsor investigator will be promptly notified.
- TAK-659 should be held and an evaluation should be conducted to correct other possible causes (eg, electrolyte disturbance, concomitant medication).
- Additional ECGs may be performed at more frequent intervals until repeated QTc measurements fall or are below the threshold interval that triggered the repeat measurement.

The decision whether to reinitiate TAK-659 treatment with or without dose reduction and additional monitoring in those patients who had asymptomatic prolonged QTc>500 msec (Grade 3) that has reverted to an acceptable interval, have previously tolerated TAK-659, and appear to have benefitted from TAK-659 treatment with either disease control or response will be agreed to by the local investigator and the sponsor investigator on a case-by-case basis.

The ECGs performed should be reviewed by the local investigator or his/her delegate before the patient leaves the clinic on visit days.

Hematology
Will include: Hemoglobin; Leukocytes with differential; Hematocrit; Neutrophils (absolute neutrophil count [ANC]); Platelet (count); Percentage of leukemic blast cells

Serum Chemistry
Will include: Blood urea nitrogen (BUN); Albumin; Calcium; Alkaline phosphatase; Chloride; Creatinine; Carbon dioxide (CO_2); Bilirubin (total) Aspartate aminotransferase (AST); Alanine aminotransferase (ALT); Uric acid; Magnesium; Lactate dehydrogenase (LDH); Amylase; Lipase; Total protein; Gamma glutamyl transferase (GGT); Glucose; Phosphate; Sodium; Potassium

Urinalysis
Turbidity and Color; Ketones; Urobilinogen; pH; Bilirubin; Glucose; Specific gravity; Occult Blood; Leukocytes; Protein; Nitrite

Ophthalmic Exam
A slit lamp eye examination will be performed by an ophthalmologist at Screening; on Cycle 2 Day 1; on Cycle 7 Day 1; every 6 cycles thereafter (+/- 2 weeks); and at the Safety follow up. On the basis of nonclinical toxicology findings with TAK-659 in rats, slit lamp examinations should focus on detecting any posttreatment changes in ocular lens. Examination and photographing of the retina will be performed at baseline but not during the study unless clinically indicated. Additional eye exams may also be performed, as required. Additionally, patients will be carefully monitored for eye complaints at each visit and instructed to report visual symptoms as soon as they occur.

8.2 Disease Assessment

Assessment of disease response will follow the criteria outlined in the Revised Recommendations of the International Working Group (IWG) for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. (35) Investigators are encouraged to consult the reference for more detailed explanation of response criteria.

CR, CRi/CRp, and PR are defined using the following criteria:

Morphologic CR:
- A CR designation requires that the patient achieve the morphologic leukemia-free state and have an ANC of more than 1,000/uL and platelets of > 100,000/uL.
- A morphologic leukemia-free state requires less than 5% blasts in an aspirate sample with marrow spicules and with a count of at least 200 nucleated cells.
- There should be no blasts with Auer rods.
- Hemoglobin concentration or hematocrit has no bearing on remission status, although the patient must be independent of transfusions.
- There should be no residual evidence of extramedullary leukemia.

Morphologic CRi, CRp:
After chemotherapy, some patients fulfill all of the criteria for CR except for residual neutropenia (<1,000/uL) or thrombocytopenia (<100,000/uL).

PR:
- This designation requires all of the hematologic values for a CR but with a decrease of at least 50% in the percentage of blasts to 5% to 25% in the bone marrow aspirate. Thus, if the pretreatment bone marrow blast percentage was 50% to 100%, the percentage of blasts must decrease to a value between 5% to 25%; if the pretreatment blast percentage was 20% to less than 49%, they must decrease by at least half to a value of more than 5%.
- A repeat bone marrow aspiration after several weeks may be required to distinguish between a PR and increased blasts caused by bone marrow regeneration.
- A value of < 5% blasts may also be considered a PR if Auer rods are present.

Progressive Disease:
Because the IWG criteria for AML do not provide a standardized definition for progressive disease, in this protocol, progressive disease is defined as 1 of the following:

- >50% increase in bone marrow blasts from baseline value.
- >50% increase in circulating blasts from baseline value with absolute blast count> 1000/mm^3
- Development of biopsy-proven extramedullary disease, or new sites of extramedullary leukemia.
- Note: If the initial marrow blast percentage is too high to base progression on a >50% increase in bone marrow blasts, then peripheral blood criteria will be used.

8.3 Bone Marrow Biopsy and Aspirate Collection Analysis

If prior results are not available, bone marrow biopsy and aspirate collection at screening will be required to assess disease burden, and exclusively from aspirate: cytogenetics, karyotype, mutations by NGS and Flt3 mutations by quantitative PCR and cellular composition by flow cytometry. Both of these tests should be done by CLIA- and/or FDA-approved vendors as standard of care. In the case of NGS testing, preference would be to have results obtained from Foundation One, Mayo, Tempus, Quest, ARUP or an institutional CLIA-certified laboratory, rendering results for a least 27 AML/MDS driver genes. If bone marrow aspiration has failed, a peripheral blood sample with circulating blasts may be substituted.

A minimum 27 gene mutational profile standard in practice for tracking mechanism and prognosis (1,3):
AML Mutations by NGS (ASXL1, CEBPA, DNMT3A, ETV6/TEL, FLT3, HRAS, IDH1, IDH2, KIT, KRAS, MLL, NPM1, NRAS, PHF6, RUNX1, TET2, TP53, WT1)
MDS Mutations by NGS (ASXL1, ATRX, BCOR, BCORL1, ETV6/TEL, DNMT3A, EZH2, GNAS, IDH1, IDH2, RUNX1, SF3B1, SRSF2, TET2, TP53, U2AF1, ZRSR2).
MPN Mutations by NGS (ASXL1, BRAF, CALR, CSF3R, EZH2, IKZF1, JAK2, JAK3, KDM6A, KIT, MPL, PDFGRA, SETBP1, TET2)
(the latter two panels have certain targets that apply more commonly to secondary AMLs)

Additional bone marrow aspirates (remaining aspirate material from the first pull or a second or third pull of bone marrow aspirate) and biopsy specimens (segments of the first core biopsy or additional core biopsies) will also be required at screening and during the study for biomarker analysis.

However, during phase 2, bone marrow aspirate and biopsy is a screening (baseline) procedure needed for the purpose of enrollment into the trial for both disease assessment and biomarker studies. In particular, bone marrow aspirate (BMA) or blood (if BMA not available) is needed to confirm the FLT-3 mutation status in marrow blasts, and therefore essential for patient assignment to either the FLT-3-ITD or WT group, as well as to determine the mutational burden by NGS for cooperating mutant epigenetic effectors. In cases for which the performance of this baseline procedure is difficult based on an individual patient's condition and a bone marrow aspirate/biopsy test has been performed within 28 days from Day 1 of Cycle 1, the site is encouraged to discuss the case with the Sponsor Investigator or delegate. In this circumstance, the site is required to investigate whether adequate residual material from the last bone marrow aspirate procedure remains available for collection and whether the tissue processing procedures for these remaining samples conform to the requirements of this trial for the

purpose of biomarker evaluation. If so, these samples should be collected and sent to the study-designated laboratory(ies).

- Note that a bone marrow biopsy is required only at screening. After screening, a biopsy that allows more bone marrow tissue to be examined should be performed (eg, when spicules are absent from the aspirate sample or a dry tap occurs) per local institutional practice at the time of disease assessment.

In addition, Phase I bone marrow aspirate and/or biopsy specimens (if needed) will be collected to assess pharmacodynamics (PD) at day 4 or day 8 (based on tempo of peripheral blood blast reduction), and to assess disease response between Days 15 and 21 and at the end of Cycle 1, Cycle 2, and Cycle 4, provided that the disease assessment is available before Day 1 of the following cycle during dose-escalation. The Cycle 2 marrow during dose-escalation/phase I is needed to gauge impact of marrow findings on dose-modifications if patients are not routinely in remission at this stage.

In the Phase II, best response will be graded on marrow on day 22 of Cycle 3, when indicated by the trend of prior assessments, in order to document the status of composite CR, or otherwise between days 15-21 if that milestone does not appear imminent. At Cycle 2 or 4 bone marrow is not required unless cytopenias demand clarification of persistence of marrow blasts. Beyond Cycle 4, bone marrow aspirate and/or biopsy assessments (if needed) will be performed as clinically indicated based on changes in peripheral blood counts, or when it is needed to establish either CR or disease progression. At any of these time points, if adequate residual material remains, it should be sent to the study-designated laboratory(ies) for exploratory biomarker studies.

Bone marrow aspirates and/or biopsies will be collected during Phase II for biomarker studies at the time of relapse in patients who have had initial response to TAK-659/Ixazomib.

- If a bone marrow aspirate and/or biopsy is performed per standard of care based on suspected progression, if adequate residual material remains, these samples should be sent to the study-designated laboratory(ies) after discussion with the sponsor investigator to ensure the usability of the samples (whether conforming to the required tissue processing procedures for this study).
- If the patient has initially responded and subsequently shows clinical signs of relapse but a bone marrow aspirate and/or biopsy have not been obtained, the site will collect a bone marrow aspirate and/or biopsy at this time to assess disease. During the Procedure, additional bone marrow aspirates (remaining aspirate material from the first pull or a second or third pull of bone marrow aspirate) and biopsy specimens (segments of the first core biopsy or additional core biopsies) will be collected for biomarker studies and sent to the study-designated laboratory(ies).

Details regarding the preparation, handling, and shipping of these samples are provided in the Correlative Laboratory Manual.

8.4 Pharmacokinetic Measurements

The primary aim of PK sampling in this study is to measure the plasma concentrations of TAK-659 when given in tandem with Ixazomib. It is not intended that these measurements be intensive, but all patients (Phase I and Phase II) should have the following samples drawn:

After the morning dose:

Cycle 1 Day 1 (predose, 2-4 hour postdose)

Cycle 1 Day 15 (predose, 2-4 hour post dose)

Cycle 2 Day 15 (predose, a postdose sample any time during the clinical visit)

Cycle 3 Day 15 (predose, a postdose sample any time during the clinical visit)

Cycle 4 Day 15 (predose, a postdose sample any time during the clinical visit)

However, plasma collected for TAK-659 PK measurements may additionally be used for exploratory measurement of metabolites of TAK-659, if technically feasible and considered necessary for further understanding the metabolism and clearance of TAK-659. Details on the collection, storage, processing, handling, and shipping of the PK samples are provided in the Laboratory Manual. The evaluable pharmacokinetic population would be those patients whom have at least one measurable post TAK-659 dose concentration.

8.5 Phase II Interim Analysis

An interim analysis for futility stopping will be conducted to determine whether continuation of the Phase II study is warranted. The investigator-assessed response rate will be used as the endpoint for the interim analysis. The FLT-3 mutant and FLT-3 WT cohorts will be evaluated independently and could be subject to enrollment hold by individual cohort.

For the FLT-3 mutant cohort, the interim analysis will be performed when the first 8 response-evaluable patients have completed 3 cycles of therapy or have discontinued therapy before 3 cycles. If more than 2 responders are observed out of these initial 8 evaluable patients, patient enrollment will continue. The number of evaluable patients needed in the second stage is 12.

For the FLT-3 WT cohort, the interim analysis will be performed when the first 9 response-evaluable patients have had the opportunity to complete up to 3 cycles of therapy or have discontinued therapy before 3 cycles. If more than 1 responder is observed out of these initial 9 evaluable patients, patient enrollment will continue. The number of evaluable patients needed in the second stage is 7. These parameters were developed in context of the Statistical Methods described in Section 13.

8.6 Safety Follow-up Evaluations

A safety follow-up visit should occur when subjects permanently stop study treatment for whatever reason (toxicity, progression, or at discretion of site investigator) and should be performed 30 days (±7 days) after the last dose of treatment. Subjects who have an ongoing ≥ grade 2 or serious AE (SAE) at this visit will continue to be followed until the AE resolves to ≤ Grade 1 or baseline, is deemed clinically insignificant, and/or until a new anti-cancer treatment starts, whichever is earlier.

8.7 Long Term Follow-up Evaluations

All patients, including those patients no longer on treatment, will be assessed for survival. Patients who discontinue the study, regardless of reasons for discontinuation, will be followed for survival every month for up to 12 months after discontinuation of the study drug or until death, loss to follow-up, or withdrawal of consent for further follow-up, whichever comes first. In addition, information on any subsequent anticancer therapies will be collected during the survival follow-period. For patents who achieve CR but discontinue study treatment while still in remission, disease progression data will also be collected during the survival follow-up period.

Follow up may be accomplished via clinic visit, phone call, or other avenues as appropriate.

9. BIOSPECIMEN STUDIES AND PROCEDURES

This trial informs an overriding impasse to prevent progress in therapy of AML. That impasse results from the fact that a majority of AMLs are of high risk for failure of induction chemotherapy, or subject to early relapse, due to intrinsic genetic and epigenetic features.

Unfortunately, these general features are quite diverse and yet an inroad against each scenario is almost always specific to the existence of a combination of 2 or 3 of such mutations within each case. This applies to the majority of AMLs of normal karyotype or those with nonrecurring cytogenetic changes, as opposed to AMLs with complex karyotype. Complex karyotype AMLs have the more profound defect for TP53 mutation that belies easy remedy.

On the other hand, those normal karyotype AMLs are characterized by vulnerability to targeting agent combinations that unravel epigenetic silencing or enhancement of those participants in a final common pathway to therapeutic resistance embodied by HoxA overexpression, which is imparted by the combination of 2 or 3 individual mutations, as noted in the Rationale.

Indeed, we and others have pursued and identified the existence of ameliorative interventions against this hurdle to sensitivity for both tyrosine kinase inhibitors and chemotherapy, and, in fact, both inhibition of Syk and application of proteasome inhibitors are a pathway to such sensitization 2,7,15,17, 18). Bortezomib, either alone, or in our prior combination with Sorafenib, Vorinostat, was found to sharply curtail HoxA expression, leading to sensitivity. Mechanisms identified within these remedies included 1) prevention of proteasomal degradation of EZH2, thus leading to reengagement of PRC2-mediated repression of HoxA9 (2); 2) AMLs with Meis1 overexpression are 3x more sensitive to Syk inhibition in vitro, leading in vivo to prolonged survival in animals with xenografts (17). In our study, Bortezomib in combination with Vorinostat/Sorafenib, led to strong downmodulation of HoxAs, following upon de-repression of RUNX3, an antagonist of the HoxA pathway, and sharp downregulation of Id1, a HoxA pathway agonist. In our study, the efficacy of the combination was significantly linked to co-occurrence of mutations of Flt3(ITD) and TET2 (7).

It is our hypothesis that the novel Flt3/Syk inhibitor **TAK-659 is key to further sensitization** in AMLs described by a combination of mutations that drive high HoxA and Meis1 expression, such as **category 1 mutations:** Flt3ITD/TKD,KRAS, and mNPM1, as well as mutant JAK2, when they combine with **category 2 mutations:** either IDH1/2, TET2, ASXL1, or WT1 (mutually exclusive with TET2 mutation conferring the same phenotype as mutation of its binding partner, TET2)(Ref 22). Mutations within all of these components feed into the final common pathway for WNT, affecting repression of WNT antagonists, such as RUNX3. **Efficacious intervention involving TAK-659 and Ixazomib would be expected to interrupt the pathway to HoxA/Meis1 via RUNX3 upregulation and dampened β-catenin (see ref 2, ref 7 and ref 17, as well as Figures 1-2, -3** (in vitro confirmation of the hypothesis regarding sensitivity)**). In addition, we have in vitro data to support the synergistic activity of TAK-659 with Bortezomib against very high risk AML with high allele burden Flt3ITD and DNMT3A mutations, which likely add an additional pathway independent of WNT to HOXA (see above).**

This sensitization by TAK-659/Ixazomib is also expected to be reflected in a broader transcriptional upregulation/derepression of the tumor suppressor gene repression cohort characterizing the sensitive AML subtypes, in addition to RUNX3 (see ref 6): CDKN2A, DAPK1(see ref 7).

We will apply statistical methods to validate these mechanistic associations which predict responsiveness and drug efficacy in patients as in our prior work (7), and that of other recent targeted therapy trials (36, 37).

Thus, the stage is set for our correlative studies to define what are the mutational combinations within normal karyotype/nonrecurring karyotype AML lending sensitivity to TAK-659 and Ixazomib. To pursue this infrastructure in detail:

1. All patients on this protocol with relapsed/refractory AML will have undergone cytogenetic studies to annotate them as AML without complex karyotype or core-binding factor mutations as exclusionary categories.

2. Patients' AML blasts will have undergone analysis by next-generation sequencing, at a minimum, for a 27-gene panel of those genes important for prognostic and functional phenotypic classification. This is a routine clinical practice served by a CLIA certified laboratory such as Foundation One, Mayo, Tempus, Quest, ARUP or an institutional CLIA-certified laboratory:
   A minimum 27 gene mutational profile standard in practice for tracking mechanism and prognosis (1):
   - **AML Mutations by NGS** (ASXL1, CEBPA, DNMT3A, ETV6/TEL, FLT3, HRAS, IDH1, IDH2, KIT, KRAS, MLL, NPM1, NRAS, PHF6, RUNX1, TET2, TP53, WT1)
   - **MDS Mutations by NGS** (ASXL1, ATRX, BCOR, BCORL1, ETV6/TEL, DNMT3A, EZH2, GNAS, IDH1, IDH2, RUNX1, SF3B1, SRSF2, TET2, TP53, U2AF1, ZRSR2).
   - **MPN Mutations by NGS** (ASXL1, BRAF, CALR, CSF3R, EZH2, IKZF1, JAK2, JAK3, KDM6A, KIT, MPL, PDFGRA, SETBP1, TET2)
   - (the latter two panels have certain targets that apply more commonly to secondary AMLs)

3. Mutational species leading to combinatorial mutational categories include: mutations of Flt3: ITD and TKD (35-40% overall), TET2 (15-20% overall), DNMT3A (40% overall), ASXL1 (10% overall), WT1 (<5% overall, 20% Flt3mutants), IDH1/2 (10-12% each among all AMLs as well as among Flt3mutants).

Translational laboratory pharmacodynamic analysis will be undertaken in the laboratory of Reuben Kapur, PhD, Associate Director of Indiana University Cancer Center (Co-Director of Hematopoiesis, Hematologic Malignancy, Immunology) and Director of Leukemia Translational Research for the IU Cancer Center. In addition, select assays will be performed in other Big Ten institution labs: Dr. Brian Parkin-digital PCR for determination of clonal minimal residual disease. Certain proteomic assays, including mass spectroscopic determination of peptide identity, including β-catenin and FOXM1 will be done by Drs. Irum Khan and Uma Aryal at University of Illinois, Chicago and Purdue University, respectively.

Cells for in vitro analyses: Bone marrow blast cells are obtained from patients within 7 days prior to treatment and at the end of 3-4 days of therapy, and processed as previously described (7).

DNA Measurements

At screening, duplicate buccal swab specimens will be obtained from patients enrolled in Phase I and Phase II. These will be used to establish the multiclonal origins of the individual AML from non-germline mutation(s) and to accurately assign allele frequency decrement(s) or not with therapy for

proper MRD assessment. Detailed instructions for the collection, processing, and shipment of samples are provided in the Correlative Laboratory Manual.

Real-time RT PCR analysis in a focused gene-set array panel
This is as described (38). qRT-PCR for 30 AML related genes and one housekeeping gene will be performed at low-density array (LDA) format according to the manufacturer's protocol (TaqMan Gene Expression Micro Fluidic card, 4346799, Applied Biosystems/Life Technologies). 18S rRNA will be chosen from TaqMan Human Endogenous Control Plate (Applied Biosystems) as internal control. Relative expression will be calculated using RQ manager Ver 1.2 (Applied Biosystems) using one-patient volunteer as a calibrator sample (fusion core-binding factor-positive (CBF+*ve*[inv(16)]), negative for *Flt3ITD*, and very low *c-jun* and *Meis-1* expression). Copy number or fold-change in expression will be calculated using the 2-ΔΔCt method.(38)

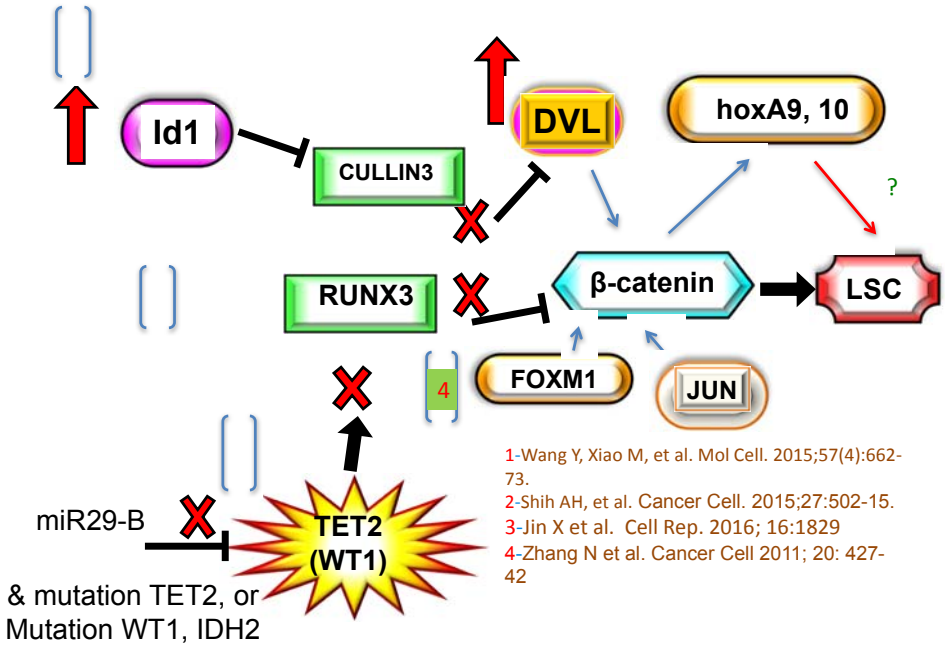
Immunoblot analysis Cytosolic or nuclear proteins will be subjected to Western blotting with indicated antibodies as described. (7)

Responders will be annotated with this information as to the AML genetic and epigenetic phenotype, as well as into categories of pathways involved as in our prior study, using those statistical association platforms.

Hypothesis: Experimentation will be performed to further elucidate the validity of an hypothesis generated within the prior combination targeted therapy with Bortezomib, Sorafenib, Vorinostat. That hypothesis holds that Flt3 mutant AML requires collaboration from among mutant epigenetic modifiers: 1) that selectively act within the Wnt pathway in order to disrupt antagonists to β-catenin-the end-effector of this pathway en route to HOXA, including RUNX3; or 2) they may have more direct action on DNA/chromatin methylation density of specific gene effectors, particularly HOXA's, that leads to an upregulation of this crucial gene's expression. Examples of the former mutationally affected epigenetically-active enzymes required for cooperation with Flt3mutants are: WT1, IDH1/2, TET2. Example of the latter is mutant DNMT3A.

A corollary hypothesis is that Syk is a necessary collaborator in leukemic transformation in that its activity is required for expression of MEIS1, which is necessary for direct collaboration with HOXA to promote leukemic transformation. Thus, utilization of the dual Flt3/Syk inhibitor TAK-659 is crucial to abrogation of both HOXA and MEIS1, and, as a result, the leukemic endgame of progression and therapeutic resistance.

The fact that proteasome inhibitors such as Bortezomib and Ixazomib can also lead to prevention of proteasomal degradation of the tumor suppressor EZH2, in order to engage HOXA repression by PRC2, while, at the same time, lead to Flt3 protein degradation through autophagy, is a necessary reinforcement of the prior set of events toward clonal extinction of the leukemic phenotype. Additionally, FOXM1 is a required chaperone for nuclear entry of β-catenin, and has recently been demonstrated a proteasome inhibitor target via HSP70 upregulation and capture for inactivation



Testing of RNA and protein (cytosolic and nuclear) of the recovered blast cells from the patient at inception of the study, and at 4 days into the therapy, will provide direct answers about mechanisms required to transpire in the interval of time 0 to early response (day 4+) required for remission to evolve. As suggested in the diagram, are the following specific questions to be asked.

Mechanistic Studies of Required Pathways for drug activity (Indeed, understanding the mechanism will allow further modulation by additional epigenetically-active agents toward optimal responsiveness for chemotherapy sensitization)

#1. Is extinguished ID1 expression an early required event en route to remission, and does this feed into the pathway of β-catenin downregulation because ID1 is no longer able to repress CULLIN3, thus blunting Wnt pathway activation and further resulting in downregulation of the DVL/JUN complex to β-catenin activity?

Approach: CULLIN levels in protein lysates will be tested, along with DVL/JUN/β-catenin complexes by immunoprecipitation and immunoblotting in conjunction with determination of ID1mRNA levels in marrow blasts, before and at day 4 or 8 into therapy.

#2. Is RUNX3 mRNA de-repression also required for early steps in remission by way of its role in RUNX3 protein's availability for binding and antagonizing β-catenin, thus preventing TCF4 target transcriptional activation (c-myc, cyclin D1, HOXA)?

Approach: Corresponding with determination of RUNX3 mRNA upregulation during early initiation of therapy, RUNX3 protein levels will be determined in cytosolic and nuclear lysates, and compared with simultaneous existence of β-catenin in those distinct compartments by immunoprecipitation and immunoblotting. We will expect that heightened RUNX3 expression will sequester β-catenin in the

cytoplasm. We will also determine in subsequent cases described by the same events related to RUNX3 upregulation, whether promoters of TCF4/β-catenin target genes are, as a result, now devoid of these transactivating proteins JUN/β-catenin by ChIP.

#3. Are there circumstances where treatment-induced RUNX3 mRNA upregulation does not occur, however, early during therapy it is observed that Wnt target genes are abrogated? This might occur by virtue of TAK-659 activity to prevent PAK1-mediated phosphorylation of resident RUNX3 (a binding partner of PAK1), thus preventing its sequestration/inactivation of β-catenin.

Approach: RUNX3 and β-catenin compartment localization will be studied, and the autonomous phosphorylation of threonine 209 of RUNX3 in untreated cells will be correlated with the demonstrated apartheid of RUNX3 from β-catenin in distinct cellular compartments, which, under control conditions, would ensure β-catenin availability in the nucleus to bind to TCF4-dependent target promoters. It is expected that treatment will reverse this state, dependent upon loss of TK-to-PAK1 activation and its RUNX3 phosphorylation.

#4. Is there a required mechanistic interaction between FOXM1 and β-catenin that explains dramatic synergy of TAK-659 with proteasome inhibitor Ixazomib? Indeed, it is known that proteasome inhibitors degrade FOXM1 in several cancers and that FOXM1 is a necessary chaperone for β-catenin on Wnt/TCF4 target gene promoters, such as HOXA9/10. This proteasome inhibitor activity against FOXM1 appears to occur as a result of upregulation of HSP70, which binds and sequesters FOXM1 protein from positively autoregulating its promoter. The inactivation of FOXM1 leads to loss of nuclear transport of β-catenin destined for Wnt/TCF4 promoters.

Approach: We will perform reciprocal immunoprecipitations of cytosolic and nuclear proteins from lysates of patient blasts prior to and at 4 days into therapy by FoxM1 and β-catenin antibodies. Subsequent experiments will look at TCF4 elements of the β-catenin or HoxA10 promoter for co-localization at these timepoints (DNA binding activity of FOXM1itself has been demonstrated not to be required in the former) by ChIP.

#5. Alternatively, does the activity of proteasome inhibitor Ixazomib in our cases derive from its prevention of active proteasomal degradation of EZH2, which is not mutated, and is overexpressed by TK signals like mutantFlt3. This overexpressed and unmutated EZH2, when posttranslationally modified is sensitized to proteasomal degradation, which has been demonstrated to be prevented by Bortezomib in AML cells as described in the Rationale.

Approach: We will isolate nuclear and cytosolic protein from patient blasts prior to and following initiation of therapy and perform immunoprecipitation of EZH2 using both direct antibodies as well as anti-ubiquitin antibodies to determine EZH2 upregulation. This will be correlated with remission success. Subsequent experiments in the human Flt3 mutant cell line, Molm14, which has demonstrated cooperative sensitivity to TAK-659 and Bortezomib in our lab, will be used to pilot ChIP experiments for the HOX locus to identify re-engagement of PRC2 in the process of HOX repression following therapy. This will be translated to select patient samples in the process of treatment.

#6. To what degree is sensitivity to TAK-659/Ixazomib demonstrable in Flt3 wild-type AML phenotypes, and is that sensitivity displayed in context of documented epigenetic mutations (category 2

genes) enabling the Wnt pathway in the setting of a paired mutation of category I mutations in the TK pathway downstream, such as KRAS, JAK2, NPM1 gene(s), etc.

Approach: Statistical association of remission status imparted by TAK-659/Ixazomib among these paired categories (specific category 1 with category 2 drivers) will be sought.

#7. What is the depth of clonal reduction that describes TAK-659/Ixaxomib-induced remissions within Flt3mutant and Flt3 WT cases, and what is the best biomarker for measurement of clonal reduction? In the two scenarios (WT or mutant), is a category 1 mutant marker or category 2 mutant marker/driver gene better representative of the clonal reduction?

Approach: In patient marrows followed in sequential cycles of remission induction with TAK-659/Ixazomib, we will assess the clonal depth of remission by digital PCR (5-log sensitivity) of variant allele frequency for mutant driver genes from category1 and category 2 gene participants, using primers created from their mutant sequence at inception of therapy, as described by our Big Ten colleague Dr. Brian Parkin at University of Michigan. This will be a direct demonstration of the possible routes to remission: coordinate elimination of TK and epigenetic driver mutants, vs. one class driver vs. the other, existing among different clonal populations in the diseased patient. In particular, given the different mechanistic impact of Wnt pathway epigenetic genes (TET2 vs. IDH1/2 vs. WT1) vs. DNMT3A, this analysis may also help identify what category of disease may benefit from addition of another drug class to the combination in future investigations.

Overall, these supplemental exploratory studies are crucial to better understanding of the upstream events prior to HOXA/MEIS1 downregulation that the agent combination targets. Later experiments will look at the HOXA/MEIS1 target genes that exert drug resistance, including those that mediate DNA damage-repair, whose downregulation may not only sensitize to chemotherapy, but also PARP inhibitors.

9.1 Source and Timing of Biospecimen Collections

Details on the collection, storage, processing, handling, and shipping are provided in the Laboratory Manual.

Plasma for Pharmacokinetic Measurements
Phase 1 and Phase 2

- Cycle 1 Day 1 (predose, 2-4 hour postdose)
- Cycle 1 Day 15 (predose, 2-4 hour post dose)
- Cycle 2 Day 15 (predose, a postdose sample any time during the clinical visit)
- Cycle 3 Day 15 (predose, a postdose sample any time during the clinical visit)
- Cycle 4 Day 15 (predose, a postdose sample any time during the clinical visit)

Plasma collected for TAK-659 PK measurements may additionally be used for exploratory measurement of metabolites of TAK-659, if technically feasible and considered necessary for further understanding the metabolism and clearance of TAK-659.

Bone Marrow Aspirate for Biomarker Analyses

Bone marrow aspirate will be collected for translational laboratory pharmacodynamic analysis, qPCR, in vitro analyses, proteomic assays via Western blotting, and MRD via digital PCR. Bone marrow aspirate samples will be collected at the following timepoints:

Phase 1

- Screening,
- Cycle 1 Day 4 or Day 8: Day 4 collection for the 1st 5 patients. Depending on timing of index peripheral blood reduction, collection may be moved to day 8 for subsequent patients.
- Cycle 1 Day 15
- Cycle 2 Day 15
- Cycle 4 Day 15

Phase 2

- Screening
- Cycle 1 Day 4 or Day 8: depending on findings from Phase I patients.
- Cycle 1 Day 15
- Cycle 3 Day 22 (or within 3 days prior to C4D1)*
- Progression

Note: In cases when insufficient bone marrow aspirate is collected, peripheral blood will be collected and submitted per the instructions in the CLM.

****In the case of post-Cycle 3 assessment, aspirate and biopsy are suggested on day 22 (or within 3 days prior to C4D1), simultaneous with the plan to continue onto Cycle 4 day 1 that same day following the procedure, so to serve as record establishing the optimal response/composite CR. Such planning should be guided by the trend of blast reduction observed during Cycles 1, 2.***

Buccal Swabs for Germline DNA

Phase 1 and Phase 2

- Pre-treatment Cycle 1 Day 1

Serum for Proteomic Analysis

Phase 1 and Phase 2

- Pre-treatment Cycle 1 Day 1
- Cycle 1 Day 4 or Day 8: collection day will correspond to BM aspirate

9.1.1 Banking of Leftover Biospecimens

Subject consent will be obtained to bank any leftover samples collected for study-specific correlative research. Hoosier Cancer Research Network (HCRN), as Administrative Headquarters for the Big Ten CRC, will manage the banked samples. Samples will be banked indefinitely in the Hoosier Cancer Research Network Biorepository and used for future unspecified cancer-related research.

9.1.2 Banking Samples for Future Unspecified Research

Subject consent will be obtained to collect additional samples for future unspecified Big Ten Cancer Research Consortium studies. HCRN will manage the banked samples. Samples will be banked indefinitely in the HCRN Biorepository.

This includes:

- Whole blood: Whole blood will be collected prior to treatment on Cycle 1 Day 1.
- Pre- and Post-treatment plasma: Whole blood for plasma will be collected prior to treatment on Cycle 1 Day 1 and at the 30-day Safety Follow-up visit.
- Pre- and Post-treatment serum: Whole blood for serum will be collected prior to treatment on Cycle 1 Day 1 and at the 30-day Safety Follow-up visit.

Please refer to the Correlative Laboratory Manual (CLM) for all sample collection, processing, labeling, and shipping instructions.

9.1.3 Confidentiality of Biospecimens

Samples will be identified by a subject's study number assigned at the time of registration to the trial. Any material issued to collaborating researchers will be anonymized and only identified by the subject's study number.

10. CRITERIA FOR DISEASE EVALUATION

Assessment of disease response will follow the criteria outlined in the Revised Recommendations of the International Working Group (IWG) for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. (34) Investigators are encouraged to consult the reference for more detailed explanation of response criteria.

CR, CRi/CRp, and PR are defined using the following criteria:

Morphologic CR: A CR designation requires that the patient achieve the morphologic leukemia-free state and have an ANC of more than 1,000/uL and platelets of $\geq$ 100,000/uL. A morphologic leukemia-free state requires less than 5% blasts in an aspirate sample with marrow spicules and with a count of at least 200 nucleated cells. There should be no blasts with Auer rods. Hemoglobin concentration or hematocrit has no bearing on remission status, although the patient must be independent of transfusions. There should be no residual evidence of extramedullary leukemia.

Morphologic CRi, CRp: After chemotherapy, some patients fulfill all of the criteria for CR except for residual neutropenia (<1,000/uL) or thrombocytopenia (<100,000/uL).

PR: This designation requires all of the hematologic values for a CR but with a decrease of at least 50% in the percentage of blasts to 5% to 25% in the bone marrow aspirate. Thus, if the pretreatment bone marrow blast percentage was 50% to 100%, the percentage of blasts must decrease to a value between 5% to 25%; if the pretreatment blast percentage was 20% to less than 49%, they must decrease by at least half to a value of more than 5%. A repeat bone marrow aspiration after several weeks may be required to distinguish between a PR and increased blasts caused by bone marrow regeneration. A value of $\leq$ 5% blasts may also be considered a PR if Auer rods are present.

Progressive Disease

Because the IWG criteria for AML do not provide a standardized definition for progressive disease, [12] in this protocol, progressive disease is defined as 1 of the following:

- >50% increase in bone marrow blasts from baseline value.
- >50% increase in circulating blasts from baseline value with absolute blast count> 1000/mm^3
- Development of biopsy-proven extramedullary disease, or new sites of extramedullary leukemia.

Note: If the initial marrow blast percentage is too high to base progression on a >50% increase in bone marrow blasts, then peripheral blood criteria will be used.

11. DRUG INFORMATION

11.1 TAK-659

11.1.1 Supplier/How Supplied

TAK-659 has been formulated into immediate-release film-coated tablets for use in phase 1 clinical studies via a common granulation process. Three different tablet dosage strengths, 20 mg, 60 mg, and 100 mg, were formulated. The formulation contains compendial excipients that include mannitol, microcrystalline cellulose, hydroxypropyl cellulose, sodium starch glycolate, and magnesium stearate. Tablets were coated with Opadry® film coat.

TAK-659 20 mg, 60 mg, and additional dose strength tablets will be packaged into round, white, high-density polyethylene (HDPE) bottles with induction seal, desiccant pack, and polypropylene child resistant caps. Each bottle containing 30 tablets of TAK-659 will be labeled with either a single-panel or multi-language label containing pertinent study information, country-specific requirements, and a caution statement.

Takeda will supply TAK-659 at no charge to subjects participating in this clinical trial.

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution, and usage of investigational product in accordance with the protocol and any applicable laws and regulations.

11.1.2 Storage and Stability

TAK-659 tablets should be stored in the original dispensing bottles at 1°C to 25°C (33.8°F-77°F) with excursions permitted to 30°C (86°F) as long as they do not exceed 7 days. All temperature excursions of the tablets must be reported back to the manufacturer for assessment and determination for continued use. The TAK-659 tablets must be used before the retest date indicated on the label and/or accompanying documentation. Throughout the duration of the clinical trial, the stability of the drug product will be monitored. TAK-659 tablets should remain in the original bottle provided to the investigational TAK-659 site and patients. Drug supply must be kept in an appropriate, limited access, secure place until it is dispensed to the enrolled patients.

Since TAK-659 is an investigational agent, it should be handled with due care. In the case of broken tablets, raising dust should be avoided during the clean-up operation. Damaged tablets may be harmful by inhalation, ingestion, or skin and/or eye contact. In the case of contact of damaged tablets with the eyes or skin, there should be immediate and thorough flushing and washing for at least 15 minutes with water (and soap for skin). Medical personnel should be notified.

Patients are to be instructed on proper storage, accountability, and administration of TAK-659, including that TAK-659 is to be taken as intact tablets.

A study drug accountability log must be completed for all study drug dispensed and administered to study patients.

11.1.3 Handling and Disposal

Patients should be instructed to return their empty blister packs to the investigative site, rather than discarding them. Reconciliation will occur accordingly when the patient returns for their next cycle of take-home medication.

11.1.4 Dispensing

TAK-659 must be dispensed only from official study sites and to eligible subjects under the supervision of the site investigator.TAK-659 must be stored in a secure area according to local regulations. The investigative site is responsible for dispensing TAK-659 in the correct daily dose configurations. Comprehensive instructions should be provided to the patient in order to ensure compliance with dosing procedures. Patients who are receiving take-home medication must be given only 1 cycle of medication at a time.

11.1.5 Packaging and Labeling

TAK-659 20 mg, 60 mg, 100 mg, and additional dose strength tablets will be packaged into round, white, high-density polyethylene (HDPE) bottles with induction seal, desiccant pack, and polypropylene child resistant caps. Each bottle containing 30 tablets of TAK-659 will be labeled with either a single-panel or multilanguage label containing pertinent study information, country-specific requirements, and a caution statement.

11.1.6 Adverse Events

See summary of adverse events in section 1.4.6. Please also reference the current TAK-659 IB for a comprehensive list of adverse events.

11.2 Ixazomib

Ixazomib is a reversible proteasome inhibitor. Ixazomib preferentially binds and inhibits the chymotrypsin-like activity of the beta 5 subunit of the 20S proteasome.

Ixazomib induced apoptosis of multiple myeloma cell lines in vitro. Ixazomib demonstrated in vitro cytotoxicity against myeloma cells from patients who had relapsed after multiple prior therapies, including bortezomib, lenalidomide, and dexamethasone. The combination of ixazomib and lenalidomide demonstrated synergistic cytotoxic effects in multiple myeloma cell lines. In vivo, ixazomib demonstrated antitumor activity in a mouse multiple myeloma tumor xenograft model.

11.2.1 Supplier/How Supplied

Ixazomib will be supplied by Takeda Oncology at no charge to patients participating in this clinical trial. The ixazomib drug product will be from investigational supply.

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution, and usage of investigational product in accordance with the protocol and any applicable laws and regulations.

11.2.2 Description of Investigational Agent

The ixazomib drug product is provided in strengths of 4.0-, 3.0-, and 2.3-mg capsules as the active boronic acid. The dose strengths are differentiated by both capsule size and color as described below:

Dose Strength	Capsule Size	Capsule Color
4.0 mg	Size 4	Ivory
3.0 mg	Size 3	Light gray
2.3 mg	Size 2	Light pink

For additional details, please see the ixazomib IB.

11.2.3 Storage and Stability

Upon receipt at the investigative site, ixazomib should remain in the blister and carton provided until use or until drug is dispensed. Ixazomib should be stored at the investigative site between +2°C and +30°C (35.6°F-86°F). Do not freeze ixazomib or store above 30°C. Any extreme in temperature should be reported as an excursion and should be dealt with on a case-by-case basis.

11.2.4 Handling and Disposal

Ixazomib is a cytotoxic drug. Follow applicable special handling and disposal procedures. Do not open or crush capsules. Avoid direct contact with the capsule contents. In case of capsule breakage, avoid direct contact of capsule contents with the skin or eyes. If contact occurs with the skin, wash thoroughly with soap and water. If contact occurs with the eyes, flush thoroughly with water.

Any unused medicinal product or waste material should be disposed in accordance with local requirements.

11.2.5 Dispensing

Ixazomib must be dispensed only from official study sites and to eligible subjects under the supervision of the site investigator. Ixazomib should be stored in a secure area according to local regulations. It is the responsibility of the site investigator to ensure that study drug is only dispensed to subjects.

11.2.6 Adverse Events

The most common adverse events associated with ixazomib are thrombocytopenia, gastrointestinal toxicity (diarrhea, constipation, nausea, and emesis), peripheral neuropathy, peripheral edema, and cutaneous reactions. Please see the current IB for the comprehensive list of adverse events.

12. ADVERSE EVENTS

12.1 Definitions

12.1.1 Adverse Event (AE)

An AE is any untoward medical occurrence whether or not considered related to the study drug that appears to change in intensity during the course of the study. The following are examples of AEs:

- Unintended or unfavorable sign or symptom
- A disease temporally associated with participation in the protocol
- An intercurrent illness or injury that impairs the well-being of the subject

Abnormal laboratory values or diagnostic test results constitute AEs only if they induce clinical signs or symptoms or require treatment or further diagnostic tests

Hospitalization for elective surgery or routine clinical procedures that are not the result of an AE (e.g., surgical insertion of central line) should not be recorded as an AE.

Disease progression should not be recorded as an AE, unless it is attributable to the study regimen by the site investigator.

12.1.2 Serious Adverse Event (SAE)

An SAE is an adverse event that:

- Results in death. NOTE: Death due to disease progression should not be reported as a SAE, unless it is attributable by the site investigator to the study drug(s)
- Is life-threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- Requires inpatient hospitalization for >24 hours or prolongation of existing hospitalization. **NOTE:** Hospitalization for anticipated or protocol specified procedures such as administration of chemotherapy, central line insertion, metastasis interventional therapy, resection of primary tumor, or elective surgery, will not be considered serious adverse events.
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly or birth defect
- Is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention (e.g., medical, surgical) to prevent one of the other serious outcomes listed in the definition above). Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions not resulting in hospitalization; or the development of drug dependency or drug abuse.
- New primary malignancy
- Pregnancy

12.1.3 Unexpected Adverse Event

For this study, an AE is considered unexpected when it varies in nature, intensity or frequency from information provided in the current IB, package insert, or when it is not included in the informed consent document as a potential risk. Unexpected also refers to AEs that are mentioned in the IB as occurring with a class of drugs or are anticipated from the pharmacological properties of the drug but are not specifically mentioned as occurring with the particular drug under investigation.

12.1.4 Relatedness

AEs will be categorized according to the likelihood that they are related to the study drug(s). Specifically, they will be categorized using the following terms:

Unrelated	The Adverse Event is ***not related*** to the drug(s)
Unlikely	The Adverse Event is ***doubtfully related*** to the drug(s)
Possible	The Adverse Event ***may be related*** to the drug(s)

Probable	The Adverse Event is ***likely related*** to the drug(s)
Definite	The Adverse Event is ***clearly related*** to the drug(s)

12.2 Reporting

12.2.1 Adverse Events

- AEs will be recorded from time of signed informed consent until 30 days after discontinuation of study drug(s).
- AEs will be recorded regardless of whether or not they are considered related to the study drug(s).
- All AEs will be recorded in the subject's medical record and on the appropriate study specific eCRF form within the EDC system.
- All AEs considered related to study drug(s) will be followed until resolution to ≤ Grade 1 or baseline, deemed clinically insignificant, and/or until a new anti-cancer treatment starts, whichever occurs first.

12.2.2 Serious Adverse Events (SAEs)

12.2.2.1 Site Requirements for Reporting SAEs to Big Ten CRC Administrative Headquarters

- SAEs will be reported from time of signed informed consent until 30 days after discontinuation of study drug(s).
- SAEs will be reported on the SAE Submission Form and entered in the SAE tab in the EDC system **within 1 business day** of discovery of the event.
- SAEs include events related and unrelated to the study drug(s).
- All SAEs will be recorded in the subject's medical record and on the appropriate study specific eCRF form within the EDC system.
- All SAEs regardless of relation to study drug will be followed until resolution to ≤ Grade 1 or baseline and/or deemed clinically insignificant and/or until a new anti-cancer treatment starts, whichever occurs first.

The site will submit the completed SAE Submission Form (see the Documents/Info tab of the EDC) to Big Ten CRC AHQ within **1 business day** of discovery of the event**.** The form will be sent electronically to Big Ten CRC AHQ at safety@hoosiercancer.org. The site investigator is responsible for informing the IRB and/or other local regulatory bodies of the SAE as per local requirements.

The original copy of the SAE Submission Form and the email correspondence must be kept within the study file at the study site.

Once the SAE has resolved, sites must electronically submit a follow up SAE Submission Form within a reasonable timeframe to Big Ten CRC AHQ at safety@hoosiercancer.org.

12.2.2.2 Site Requirements for Reporting Pregnancy and Birth Events to BTCRC Administrative Headquarters

If a woman becomes pregnant or suspects that she is pregnant while participating in this study or within 90 days after the last dose, she must inform the site investigator immediately and permanently

discontinue study drug. The site must immediately submit a completed Pregnancy Form to BTCRC AHQ at safety@hoosiercancer.org. The pregnancy must be followed for the final pregnancy outcome.

If a female partner of a male patient becomes pregnant during the male patient's participation in this study (i.e. from the initiation of study drug(s) through 90 days after the last dose of study drug), the site investigator must also immediately submit a completed Pregnancy Form to BTCRC AHQ at safety@hoosiercancer.org. Every effort should be made to follow the pregnancy for the final pregnancy outcome.

12.2.2.3 Big Ten CRC AHQ Requirements for Reporting SAEs to Takeda Oncology

Big Ten CRC AHQ will report SAEs to Takeda Oncology within **1 business day** of receipt of the SAE Reporting Form from a site. Follow-up information will be provided to Takeda Oncology as reasonably requested.

SAE and Pregnancy Reporting Contact Information:
Fax Number: 1-800-963-6290
Email: TakedaOncoCases@cognizant.com

12.2.2.4 BTCRC AHQ Requirements for Reporting Pregnancy and Birth Events to Takeda Oncology

BTCRC AHQ will report all pregnancy and birth events to Takeda Oncology within **1 business day** of receipt of the Pregnancy Form. Follow-up information will be provided to Takeda Oncology as reasonably requested.

SAE and Pregnancy Reporting Contact Information:
Fax Number: 1-800-963-6290
Email: TakedaOncoCases@cognizant.com

12.2.2.5 Product Complaints or Medication Errors (Including Overdose)

A product complaint is a verbal, written, or electronic expression that implies dissatisfaction regarding the identity, strength, purity, quality, or stability of a drug product. Individuals who identify a potential product complaint situation should immediately contact Millennium (see below) and report the event. Whenever possible, the associated product should be maintained in accordance with the label instructions pending further guidance from a Millennium Quality representative.

A medication error is a preventable event that involves an identifiable patient and that leads to inappropriate medication use, which may result in patient harm. While overdoses and underdoses constitute medication errors, doses missed inadvertently by a patient do not. Investigators must record all medication errors (including overdose) on the appropriate CRF form. Individuals who identify a potential medication error situation should immediately contact Takeda (see below) and report the event.

For Ixazomib:

Phone: 1-844-N1-POINT (1-844-617-6468)
E-mail: GlobalOncologyMedinfo@takeda.com
FAX: 1-800-881-6092; **Hours**: Mon-Fri, 9 a.m. – 7 p.m. ET

For TAK-659:

Phone: 1-844-ONC-TKDA (1-844-662-8532)
E-mail: GlobalOncologyMedIfno@takeda.com
FAX: 1-800-881-6092; **Hours**: Mon-Fri, 9 a.m. – 7 p.m. ET

Product complaints in and of themselves are not AEs. If a product complaint results in an SAE, an SAE form should be completed and sent to Millennium Pharmacovigilance.

12.2.2.6 Sponsor-Investigator Responsibilities

Big Ten CRC AHQ will send a SAE summary to the sponsor-investigator **within 1 business day** of receipt of SAE Submission Form from a site. The sponsor-investigator will promptly review the SAE summary and assess for expectedness and relatedness.

12.2.2.7 Big Ten CRC AHQ Responsibilities for Reporting SAEs to FDA

For Protocols Conducted Under an IND

Big Ten CRC AHQ has been designated to manage the Investigational New Drug Application (IND) associated with this protocol on behalf of the sponsor-investigator. Big Ten CRC AHQ will cross-reference this submission to Takeda Oncology's parent IND at the time of submission. Additionally, Big Ten CRC AHQ will submit a copy of these documents to Takeda Oncology at the time of submission to FDA.

Big Ten CRC AHQ will be responsible for all communication with the FDA in accordance with 21CFR312 which includes but is not limited to the 7 and 15 Day Reports, as well as an Annual Progress Report. Additionally, Big Ten CRC AHQ will submit a copy of these reports to Takeda Oncology and IU DSMC at the time of submission to FDA.

IND Exempted Protocols

For protocols exempt from the requirements of an IND, the above stated requirements are not applicable. Big Ten CRC AHQ will continue to facilitate compliance of applicable requirements for the sponsor-investigator in relation to this study. This includes but is not limited to 21 CFR 50.20 informed consent, 21 CFR Part 56 IRB, and pertinent sections of the Public Health Service Act and FDAAA.

12.2.2.8 IND Safety Reports Unrelated to this Trial

Takeda Oncology will provide Big Ten CRC AHQ with IND safety reports from external studies that involve the study drug(s) per their guidelines. Big Ten CRC AHQ will forward the safety reports to the sponsor-investigator who will review these reports and determine if revisions are needed to the protocol or consent. Big Ten CRC AHQ will forward these reports to participating sites **within 1 business day** of receiving the sponsor-investigator's review. Based on the sponsor-investigator's review, applicable changes will be made to the protocol and informed consent document (if required). All IND safety reports will also be made available to sites via the EDC system.

Upon receipt from Big Ten CRC AHQ, site investigators (or designees) are responsible for submitting these safety reports to their respective IRBs, as per their IRB policies.

13. STATISTICAL METHODS

13.1 Study Design

Summary tabulations will be presented that will display the number of observations, mean, standard deviation, median, minimum, and maximum for continuous variables, and the number and percentage per category for categorical data.

For the phase I dose escalation study, data analyses will be primarily descriptive and graphical in nature. No formal statistical hypothesis testing will be performed.

For the Phase IIa study, ORR in the response-evaluable population will be estimated with 95% exact binomial confidence intervals (CIs), while considering the adaptive nature of Simon's two-stage design. Time-to-event data will be analyzed by the Kaplan-Meier method and results will be summarized by the 25^{th}, 50^{th}, and 75^{th} percentiles with associated 2-sided 95% CIs, as well as percentage of censored observations, by Flt3WT and mutant populations.

13.2 Sample Size and Accrual

During the dose escalation phase, dose escalation will be conducted according to a standard 3+3 dose escalation schema, and approximately 18 response-evaluable patients will be enrolled. The MTD/RP2D cohort will have at least 6 patients.

The sample sizes for the Phase II study are estimated using a one-sided test at a significance level of α= 0.1 with power of 80%. To perform the phase 2 study, we will consider a Simon's stage 2 design (See Study Overview Diagram) (39, 40). The primary objective of the phase 2 portion of the study is to detect an efficacy signal that warrants further development of the combination in AML, which will likely be annotated by molecular genetic mutational subgroups. It is most likely those will exist within the rubric of Flt3 mutation. The primary measure of efficacy for the phase 2 portion will be ORR, which will include CR, CRp, CRi, and PR. Best response will be assessed by the end of Cycle 3 of TAK-659/Ixazomib combination for the purpose of an interim analysis between Stage 1 and Stage 2. The Flt3WT and Flt3mut cohorts will proceed to the second stage if patients respond by CR, CRp, CRi.

It has been established in other trials involving single-agent Flt3-selective inhibitor(s), such as FDA-approved Gilteritinib, that a composite complete response rate of approximately 40% occurs in AML patients with Flt3 mutations (41). **However, in cases of subclonal mutant Flt3 allele burden, this may not occur, especially because the epigenetic co-mutation driver is not addressed by the TKI. In such cases, we will stratify response rates of Flt3 mutant cohorts by mutFlt3 Variant Allele Frequency (VAF) in order to address the possibility that the proteasome inhibitor Ixazomib may contribute to clonal reductions of the clones bearing epigenetic comutational driver allele as well as a subclonal mutFlt3 population, or those that lack the mutant Flt3 allele altogether.** However, despite this advantage, we would not accept markedly inferior response rates overall should they indicate a response rate less than expected for active single agent Flt3 inhibitor, and therefore set the futility threshold below 30%.

Therefore, within the Simon's two-stage design, the Flt3 mutant cohort uses as null hypothesis of response rate $\leq$ 30%, versus an alternative hypothesis of response rate $\geq$ 55%. Based on a Simon 2-stage Optimal design, 8 patients will be accrued in the first stage. If there are 2 or fewer responses in the 8

patients, the study will be stopped. Otherwise, 12 additional patients will be accrued for a total of 20. The treatment will be recommended for further study if 8 or more responses are observed in 20 patients.

On the other hand, we will follow the above rationale that proteasome inhibitor Ixazomib may contribute specific targeting of the epigenetic co-mutation driver allele in order to enhance what has been a limited contribution to antileukemic efficacy of Flt3-selective TKI's, such as Gilteritinib, as observed within subclonal Flt3 mutant as well as Flt3wild-type disease cohorts (42; see de Boer B, et al. Cancer Cell.2018;34:674-689). Indeed, the phase I/II trial for Gilteritinib reported an 8% (average of 120mg and 200 mg dose cohorts) composite Complete Response rate (CRc), and an average 16% ORR for Flt3wild-type disease which was vastly inferior to Flt3 mutant (AE Perl, et al. Lancet Oncology 18: 1061, 2017 [ref 41][see Table S8]).

Thus, there is a great need to establish platforms to target the **epigenetic co-mutation drivers of the Wnt/β-catenin pathway to HoxA, such as TET2 and WT1, as well as DNMT3A, EZH2 and ASXL1, where our in vitro evidence obtained on mutationally annotated Flt3 wild-type disease demonstrates promising activity of the combination of TAK-659 and Ixazomib.** Indeed, Syk inhibition, not simply Flt3 inhibition by the dual Flt3/Syk inhibitor appears to play an important role in such settings. Further, we have evidence that not only does TAK-659's Syk inhibitory activity target resistance mechanisms in Flt3 mutant AML, but that in combination with Ixazomib, there exists unique efficacy within the Flt3 wild-type AML's arising from the above effectors.

We will employ an initial comparator for targeted therapy futility of the combination in Flt3-WT patients, using a threshold response level similar to that derived from recent phase III trials among all-comers of Intermediate-dose cytarabine (IDAC) in different combinations, where the composite complete response (CRc) [CR, CRi, CRp] was approximately 21% among all-comers ([43,44]Roboz etal. JCO 2014, Faderl et al. JCO 2012) However, other studies find a much lower aggregate response rate equal or less than 12% by supplementation of the IDAC backbone with either gemtuzumab, liposomal daunorubicin, or by cytoxan/topotecan ([45]Litzow, etal. BrJ Haem. 2010). Thus, the initial futility screen for the Flt3WT cohort uses a null hypothesis response rate of ≤ 15% versus an alternative hypothesis of response rate ≥ 40%. Based on a Simon 2-stage Minimax design, approximately 9 patients will be accrued in the first stage. If there are 1 or fewer responses in the 9 patients, the study will be stopped. Otherwise, 7 additional patients will be accrued for a total of 16. The treatment will be recommended for further study if 5 or more responses are observed in 16 patients.

In addition, our broader goal is to later compare in exploratory fashion, the response rate among the subjects whose epigenetic mutational profile fits that profile of driver mutations acting in the Wnt-β-catenin pathway, which our combination is designed to target, to the **"investigator choice"** standard for all-comers noted above. We propose to assign a response rate among our sensitive population with the epigenetically-active mutant drivers noted above, in an attempt to validate the sensitivity paradigm we have enunciated from our in vitro testing of NGS-annotated primary blasts. Indeed, we are testing the combination whose activity links with a mutational complement of epigenetic effectors for which no targeted therapy currently exists (epigenetic co-mutation drivers of the Wnt/β-catenin pathway to HOXA, such as TET2 and WT1, as well as DNMT3A, EZH2 and ASXL1), where our in vitro evidence

obtained on mutationally annotated Flt3 wild-type disease demonstrates promising activity of the combination of TAK-659 and Ixazomib.

Other efficacy measures, such as DOR, TTP, ability of the therapy to provide bridge to transplant, and mortality rate will also be considered in the decision to expand the study. Best responses from both cohorts will be assessed individually and combined in an effort to understand the all-comer response rate. Upon enrollment, the mutational profiles from Foundation One, Mayo, Tempus, Quest, ARUP, or an institutional CLIA-certified laboratory will be recorded in the database with minimum gene coverage as noted in Section 8.3.

Retrospective analysis will be performed to identify additional patient selection markers beyond mutational profile, by scrutiny of the expression levels of mRNAs for target drivers in context of epigenetic repression of tumor suppressor genes noted in our prior published study. Particular emphasis will be paid to the reversal of a signature for hyperexpression of HOXA9/10 and MEIS1 in a final common pathway for treatment resistance that will have been reversed by upmodulation of the repressed tumor suppressors RUNX3 and/or DAPK1, while strongly dampening the agonists for β-catenin ID1, JUN, and FOXM1.

13.2.1 Toxicity monitoring for phase II study

Sequential boundaries will be used to monitor dose-limiting toxicity rate, based on the method described in Ivanova, Qaqish and Schell (Continuous Toxicity Monitoring in Phase II Trials in Oncology, Biometrics 61, 540-545, 2015[46]). This is a Pocock-type boundary where the same significance level is used for all looks. For practical implementation, we will look at the toxicity data in real time for every 3 patients, separately among the two phase II cohorts.

A DLT rate of 20% would be considered as acceptable while a DLT rate of 35% would be considered as too high and unacceptable. The type I error is chosen to be 10% and the power is chosen to be 80%. The accrual will be halted if excessive numbers of dose-limiting toxicities are seen, that is, if the number of dose-limiting toxicities is > the following boundary b out of n patients currently studied:

- for the Flt3mut cohort, the boundary will be b/n=2/3, 3/6, 4/9, 5/12, 6/15, 7/18, 7/20;
- for the Flt3WT cohort, the boundary will be b/n=2/3, 3/6, 4/9, 5/12, 5/15, 6/16.

For example, if among the first 6 patients, there are more than 3 DLTs, then the accrual for the corresponding cohort would be paused. This will be looked at separately for the Flt3 WT and mutant cohorts.

Based on this rule, for the Flt3mut cohort, if the “true” DLT rate is 20%, then the probability of early stopping is about 4.8% with expected sample size of 19.5. If the “true” DLT rate is 35%, then the probability of early stopping is about 37% with expected sample size of 16.3. For the Flt3WT cohort, if the “true” DLT rate is 20%, then the probability of early stopping is about 7.3% with expected sample size of 15.6. If the “true” DLT rate is 35%, then the probability of early stopping is about 45.7% with expected sample size of 13.6.

13.3 Randomization and Stratification

For the Phase I study, patients will be enrolled in successive dose cohorts. In the phase II portion of the study, patients will be enrolled in 2 different cohorts based upon their Flt3 mutation status. No randomization is planned for this study.

13.4 Populations for Analysis

The populations used for analysis will include the following:

- **Safety population**: Patients who receive at least 1 dose of TAK-659 and Ixazomib will be used for all safety and analyses.
- **Pharmacokinetic population**: Patients who have at least one measurable post TAK-659 dose concentration.
- **Pharmacodynamics population**: Patients with sufficient dosing in Cycle 1 and sufficient pharmacodynamics data will be used for PD analyses.
- **Response-evaluable population**: Patients who receive at least 1 dose of study drugs, have measurable disease at baseline, and 1 postbaseline disease assessment will be used for analyses of response.
- **DLT-evaluable population**: the DLT-evaluable population is defined as all patients in the phase I portion of the study who either experience DLT during Cycle 1 or complete at least 75% of the planned doses of TAK-659 and Ixazomib within 2 cycles and have sufficient follow-up data to allow the investigators and sponsor to determine whether DLT occurred.

13.5 Procedures for Handing Missing, Unused, and Spurious Data

All available efficacy and safety data will be included in data listings and tabulations. No imputation of values for missing data will be performed. The relevance of missing sample data will be assessed.

Data that are potentially spurious or erroneous will be examined according to standard data management operating procedures.

13.6 Demographic and Baseline Characteristics

Demographic and baseline characteristics will be summarized, including gender, age, race, weight, height, body surface area (BSA), primary diagnosis, and other parameters as appropriate. No inferential statistics will be carried out.

13.7 Data Analysis Plan

13.7.1 Primary Objective

Phase I portion: There is no primary efficacy endpoint for the dose escalation portion of the study. Toxicity data will be summarized and tabulated for all and for each dose level.

Phase II portion: The phase II is not intended to demonstrate differences in treatment outcomes among disease groups, although potentially informative data will be subjected to exploratory analysis. The primary endpoint is ORR. ORR is defined as the rate of CRs and PRs in the response-evaluable population. It will be estimated with 95% exact binomial confidence intervals (CIs), while considering the adaptive nature of Simon's two-stage design.

13.7.2 Secondary Objectives:

There is no secondary efficacy endpoint for the Phase I portion of the study.

Phase II study: the secondary efficacy endpoints for are ORR in FLT-3 mutant and FLT-3 WT populations, DOR, mortality rate at 3 and 6 months, and OS (by FLT-3 mutant cohort, FLT-3 WT cohort, and combined cohort). DOR is defined as the time from the date of first documentation of a response to the date of first documented progressive disease (TTP). OS is defined as the time from the date of study entry to death. ORR will be estimated with 95% exact binomial confidence intervals (Cis). Time-to-event data will be analyzed by the Kaplan-Meier method. OS at 3 and 6 months and by FLT-3 mutant cohort, FLT-3 WT cohort, and combined cohort) will be estimated using the Kaplan-Meier along with 95% CIs.

13.8 Exploratory Objectives and Pharmacokinetic Analysis

The data analyses for these exploratory objectives are mainly descriptive. We will begin such analyses by tabulating the pairwise combinations of class1/class2 mutations that occur within the Flt3- mutant vs. -wild type cohorts and summarize the frequency of these mutations (most frequent class 1 are Flt3mutation (both ITD and TKD) ~40%; vs. N/KRAS 20%); within class 2 (NPM1 40%, IDH2/IDH1 ~20%, TET2 ~15%, WT1 ~10%, ASXL1 15%). CR/CRp vs PR/nonresponse rates will be tabulated among groups based on presence of combinational number (combinational burden) and type, and will be compared by Fisher's exact test. ORR will summarized with 95%CI and PFS will be analyzed by Kaplan-Meier methods in the whole study population and in the subgroups defined by the mutation subtypes. Additionally, we will explore the noted response groups based on identified markers from our prior trial that were predictive of ability to achieve or not remission, including ID1, JUN, HOXA, as well as the currently added markers-β-catenin and FOXM1, examining their individual association with remission status using logistic regression model.

The plasma concentrations of TAK-659 will be determined by validated liquid chromatography tandem mass spectrometry (LC/MS/MS) assay methods. The pharmacokinetic population will be those patients whom have at least one measurable post TAK-659 dose concentration. Plasma TAK-659 concentrations will be summarized by time postdose, grouped by dosing schedule, dose group, and dosing cycle and day. Mean and individual plasma TAK-659 concentration data will be plotted over time, grouped by dosing schedule, dose group, and dosing cycle and day.

Sparse Plasma PK data from Phase I and Phase II will be used for the estimation of PK parameters in the PK-Evaluable Population, as noted in the prior section. Dose proportionality of TAK-659 plasma exposures will be evaluated by visual inspection of plots of individual PK parameter values versus dose. If data permits, regression analysis using a power model will also be used to assess dose proportionality.

Plasma PK data from Phase I and Phase II may be used to explore the relationship between PD markers of SYK and FLT-3 inhibition (and their downstream targets) and TAK-659 exposure (as described in Biomarker Analysis) and may be used to explore the relationship between exposure and toxicity and exposure and effectiveness using graphical and/or pharmacostatistical approaches, as permitted by the data.

TAK-659 plasma concentration-time data collected in this study, together with data collected from other studies, may contribute to population PK analysis. If applicable, the specifics of the population PK modeling approaches will be described separately in a population PK analysis plan, and the results will be reported separately from the clinical study report.

13.9 Biomarker Analysis

As noted above, the relationship between observed clinical response and candidate biomarkers will be explored to identify a biomarker(s) predictive of sensitivity and/or resistance to TAK-659/Ixazomib. Developing the potential predictive biomarker(s) of TAK-659/Ixazomib mediated antitumor activity may require analysis of data from multiple patients.

PD changes from baseline will be tabulated and summarized by patient, dose, and patient genotype, including Flt3 status and accompanying epigenetic mutations. Additionally, the relationship between changes in the PD marker and TAK-659 plasma exposure may be explored graphically and the PK/PD relationships may be explored using mathematical models as appropriate and permitted by the data. The relationship between changes in the PD marker and clinical response will also be explored.

13.10 Safety Analysis

Safety evaluations will be based on the incidence, intensity, and type of AEs; and clinically significant changes in the patient's vital signs, weight, and clinical laboratory results. Safety variables will be tabulated and presented for the safety population. Exposure to study drug and reasons for discontinuation of study treatment will be tabulated.

Treatment-emergent AEs that occur after administration of the first dose of study drug and through 30 days after the last dose of study drug, or until the start of subsequent antineoplastic therapy, whichever occurs first, will be tabulated. Treatment-emergent events will also include any AE that is considered by the investigator to be drug-related regardless of the start date of the event, or any event that is present at baseline but worsens in intensity or is subsequently considered by the investigator to be drug related.

AEs will be tabulated according to the Medical Dictionary for Regulatory Activities (MedDRA) coding dictionary for the purpose of summarization. AEs are to be tabulated using MedDRA system organ class, high-level term, and preferred term, and will include the following categories:

- Treatment-emergent AEs
- Drug-related treatment-emergent AEs
- Grade 3 or higher treatment-emergent AEs
- Grade 3 or higher drug-related treatment-emergent AEs

The most commonly reported treatment-emergent AEs (ie, those events reported by $\geq$ 10% of all patients in the safety population) will be tabulated by the MedDRA preferred term.

Tabulation also will be provided that enumerates AEs by maximum intensity. Deaths, SAEs, and AEs resulting in study drug discontinuation will be tabulated. Clinical laboratory parameters will be summarized at each scheduled time point. Shift tables will be produced for selected laboratory parameters. These tables will summarize the number of patients with each baseline NCI CTCAE grade and changes to the worst NCI CTCAE grade during the study.

Descriptive statistics for the actual values of vital signs and weight over time will be tabulated by scheduled time point.

All concomitant medications collected from screening through study period will be classified by preferred term according to the WHO drug dictionary.

Additional safety analyses may be determined at any time without prejudice to enumerate rates of toxicities and to further define the safety profile of study drugs.

13.10.1 Electrocardiogram

A summary of ECG abnormalities will be presented by visit. ECG intervals (QT, QTcB, QTcF, PR, QRS, and heart rate) will be summarized at each scheduled time point, along with change from baseline to each post treatment time point.

13.11 Interim Analysis

Due to the two stage designs of the phase II study, there will be a predefined decision rule at the end of the first stage to descide on whether the study regimen is are active enough to move on the second stage. This is not a formal interim analysis; there will be no formal interim analysis for this study. Of note, in the phase II study, the FLT-3 mutant and FLT-3 WT cohorts will be evaluated independently and could be subject to enrollment hold without affecting the other cohort.

14. TRIAL MANAGEMENT

14.1 Data and Safety Monitoring Plan (DSMP)

The study will be conducted in accordance with the Indiana University Melvin and Bren Simon Cancer Center's (IUSCC) DSMP for High Risk Phase I/II Trials.

BTCRC AHQ facilitated oversight activities for Phase I/II High Risk Trials includes:

- Review and processing of all AEs requiring expedited reporting as defined in the protocol
- Provide trial accrual progress, safety information, and data summary reports to the sponsor-investigator. For any increase in frequency of grade 3 or above adverse events (above the rate reported in the Investigator Brochure or package insert), the sponsor investigator will notify BTCRC AHQ who will notify the DSMC Chair immediately. The notification will include the incidence of study adverse events, grades, and attributions, as well as investigator statements regarding comparison with risks per the IB/ package insert.
- Notify participating sites of adverse events potentially requiring expedited reporting and subsequent DSMC recommendations for study modifications.
- Investigators will conduct continuous review of data and patient safety.
- BTCRC AHQ will coordinate weekly (Phase I) meetings (Safety Calls) or monthly (Phase II) meetings which will include representation from each accruing site.
    - These meetings should include review of data, the number of subjects and significant toxicities as described in the protocol. BTCR AHQ should maintain meeting minutes and attendance for submission to the DSMC upon request.
- Conduct the trial across all participating sites in accordance with the requirements set forth in the IUSCC DSMP.

14.2 Indiana University Data Safety Monitoring Committee Oversight

The IUSCC Data and Safety Monitoring Committee (DSMC) is responsible for oversight of subject safety, regulatory compliance, and data integrity for this trial. The DSMC will review this study to assess overall trial progress, toxicity, compliance, data integrity, and accrual per the Institutional DSMP.

Trials managed by Big Ten CRC AHQ are not routinely audited or monitored by IUSCC; however, the IUSCC DSMC retains the right to audit Big Ten CRC AHQ trials on a for-cause basis.

The IUSCC DSMC will review study data semi-annually during the active treatment and safety follow-up portion of the trial per the IUSCC DSMP.

In preparation for the IUSCC DSMC review, Big Ten CRC AHQ will provide the following:

- Monthly Summary Reports
- Reports of the following, if not already included in the Monthly Summary Report:
  - Adverse event summary report (including serious adverse events)
  - Study accrual patterns
  - Protocol deviations
- Audit and/or monitoring results, if applicable
- Data related to stopping/ dose decision rules described in study design
- Big Ten CRC AHQ Safety Call meeting minutes/ attendance

Documentation of DSMC reviews will be provided to sponsor-investigator and BTCRC AHQ. The IUSCC DSMC will notify the sponsor-investigator and other regulatory bodies, as appropriate, for issues of immediate concern. The sponsor-investigator will work with BTCRC AHQ to address the DSMC's concerns as appropriate.

At any time during the conduct of the trial, if it is the opinion of the investigators that the risks (or benefits) to the patient warrant early closure of the study, this recommendation should be made in writing to the DSMC Chair and Compliance Officer. Alternatively, the DSMC may initiate suspension or early closure of the study based on its review of the investigator reports.

14.3 DSMC DLT Review

Prior to making dose escalation/expansion/de-escalation decisions, the sponsor investigator and the Big Ten CRC AHQ study team will officially review all toxicity events for each subject for confirming treatment-related DLT. The study statistician will assist the determination of DLT and the interpretation of the statistical rule for dose escalation. Once a decision has been reached by the investigator, the official decision and toxicity data will be submitted to the DSMC via email (IUSCC-DLT-Review-L@list.iupui.edu). Treating additional subjects may not proceed until official DSMC correspondence confirms approval of dosing decisions for the next stage.

14.4 IND Annual Reports

For trials with an IND held locally by the IU principal investigator or university, the IND Annual Report will be prepared and submitted to the IUSCC Compliance Team. This report will be reviewed by the DSMC at the time of FDA submission.

14.5 Data Quality Oversight Activities

Remote validation of the EDC system data will be completed on a continual basis throughout the life cycle of the study. A summary report (QC Report) of these checks together with any queries resulting from manual review of the eCRFs will be generated for each site and transmitted to the site and the site monitor. Corrections will be made by the study site personnel.

Monitoring visits to the trial sites may be made periodically during the trial to ensure key aspects of the protocol are followed. Additional for-cause visits may occur as necessary. Source documents will be reviewed for verification of agreement with data entered into the EDC system. It is important for the site investigator and their relevant personnel to be available for a sufficient amount of time during the monitoring visits or audit, if applicable. The site investigator and institution guarantee access to source documents by Big Ten CRC AHQ or its designee.

The trial site may also be subject to quality assurance audit by Takeda Oncology or its designee as well as inspection by appropriate regulatory agencies.

14.6 Compliance with Trial Registration and Results Posting Requirements

Under the terms of the Food and Drug Administration Modernization Act (FDAMA) and the Food and Drug Administration Amendments Act (FDAAA), the sponsor-investigator of the trial is solely responsible for determining whether the trial and its results are subject to the requirements for submission to the Clinical Trials Data Bank, http://www.clinicaltrials.gov. All results of primary and secondary objectives must be posted to CT.gov within a year of completion. The sponsor-investigator has delegated responsibility to Big Ten CRC AHQ for registering the trial and posting the results on clinicaltrials.gov. Information posted will allow subjects to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and study site contact information.

15. DATA HANDLING AND RECORD KEEPING

15.1 Data Management

Big Ten CRC AHQ will serve as the Clinical Research Organization for this trial. Data will be collected through a web-based clinical research platform compliant with Good Clinical Practices and Federal Rules and Regulations. Big Ten CRC AHQ personnel will coordinate and manage data for quality control assurance and integrity. All data will be collected and entered into the EDC system by study site personnel from participating institutions.

15.2 Case Report Forms and Submission

Generally, clinical data will be electronically captured in the EDC system and correlative results will be captured in the EDC system or other secure database(s). If procedures on the study calendar are performed for standard of care, at minimum, that data will be captured in the source document. Select standard of care data will also be captured in the EDC system, according to study-specific objectives. Please see the Data and Safety Oversight Process (DSOP) guidelines for further details.

The completed dataset is housed at Big Ten CRC AHQ and is the sole property of the sponsor-investigator's institution. It should not be made available in any form to third parties, except for authorized representatives of appropriate Health/Regulatory Authorities, without written permission from the sponsor-investigator and Big Ten CRC AHQ. After the initial publication, the complete data set will be available to all Big Ten CRC institutions.

15.3 Record Retention

To enable evaluations and/or audits from Health Authorities/Big Ten CRC AHQ, the site investigator agrees to keep records, including the identity of all subjects (sufficient information to link records; e.g., hospital records), all original signed informed consent forms, copies of all source documents, and detailed records of drug disposition. All source documents are to remain in the subject's file and retained by the site investigator in compliance with local and federal regulations. No records will be destroyed until Big Ten CRC AHQ confirms destruction is permitted.

15.4 Confidentiality

There is a slight risk of loss of confidentiality of subject information. All records identifying the subjects will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available. Information collected will be maintained on secure, password protected electronic systems. Paper files that contain personal information will be kept in locked and secure locations only accessible to the study site personnel.

Subjects will be informed in writing that some organizations including the sponsor-investigator and his/her research associates, Big Ten CRC AHQ, Takeda Oncology, IRB, or government agencies, like the FDA, may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws.

If the results of the study are published, the subjects's identity will remain confidential.

16. ETHICS

16.1 Institutional Review Board (IRB) Approval

The final study protocol and the final version of the informed consent form must be approved in writing by an IRB. The site investigator must submit written approval by the IRB to Big Ten CRC AHQ before he or she can enroll subjects into the study.

The site investigator is responsible for informing the IRB of any amendment to the protocol in accordance with local requirements. In addition, the IRB must approve all advertising used to recruit subjects for the study. The protocol must be re-approved by the IRB as local regulations require.

Progress reports and notifications of adverse events will be provided to the IRB according to local regulations and guidelines.

16.2 Ethical Conduct of the Study

The study will be performed in accordance with ethical principles originating from the Declaration of Helsinki. Conduct of the study will be in compliance with ICH Good Clinical Practice, and with all applicable federal (including 21 CFR parts 56 & 50), state, or local laws.

16.3 Informed Consent Process

The site investigator will ensure the subject is given full and adequate oral and written information about the nature, purpose, possible risks and benefits of the study. Subjects must also be notified they are free

to discontinue from the study at any time. The subject should be given the opportunity to ask questions and allowed time to consider the information provided.

The subject's signed and dated informed consent must be obtained before conducting any procedure specifically for the study. The site investigator must store the original, signed informed consent form. A copy of the signed informed consent form must be given to the subject.

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