Phase Ib study of the safety and efficacy of Gemtuzumab Ozogamicin (GO) and Venetoclax in patients with relapsed or refractory CD33+ acute myeloid leukemia: Big Ten Cancer Research Consortium BTCRC-AML17-113

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PROTOCOL SIGNATURE PAGE

Phase Ib study of the safety and efficacy of Gemtuzumab Ozogamicin (GO) and Venetoclax in patients with relapsed or refractory CD33+ acute myeloid leukemia

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I confirm I have read this protocol, I understand it, and I will work according to this protocol and to the ethical principles stated in the latest version of the Declaration of Helsinki, the applicable guidelines for good clinical practices, whichever provides the greater protection of the individual. I will accept the monitor's overseeing of the study. I will promptly submit the protocol to applicable ethical review board(s).

_____ _____

Signature of Site Investigator Date

_____

Site Investigator Name (printed)

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Site Investigator Title

_____

Name of Facility

_____

Location of Facility (City and State)

PLEASE EMAIL COMPLETED FORM TO
BIG TEN CRC ADMINISTRATIVE HEADQUARTERS

SYNOPSIS

TITLE	Phase Ib study of the safety and efficacy of Gemtuzumab Ozogamicin (GO) and Venetoclax in patients with relapsed or refractory CD33+ acute myeloid leukemia
PHASE	Ib
OBJECTIVES	Primary Objective: To assess the maximum tolerated dose of Venetoclax when administered with Gemtuzumab Ozogamicin (GO) in patients with AML. Secondary Objectives: To assess the efficacy of this combination in achieving overall response rate (ORR) and anti-leukemic activity; characterize any adverse effects of GO in combination with Venetoclax; and estimate the relapse-free, event-free and overall survival. Exploratory Objectives: To assess the effects of therapy on quality of life; assess genomic minimal residual disease (MRD) to determine the kinetics of response and association with relapse-free survival.
STUDY DESIGN	Dose-escalation study (Cohorts 1-3) to determine whether there is a dose-limiting toxicity.
KEY ELIGIBILITY CRITERIA	Eligible patients, 18–75 yrs, will have relapsed/refractory AML, as defined by the World Health Organization classification; ECOG Performance score 0–2; adequate organ function; and do not meet exclusion criteria including WBC >25 × 10^9/L (hydroxyurea permitted to decrease WBC count), unresolved ≥grade 2 clinically significant nonhematologic toxicities from prior anticancer therapy or *unresolved* ≥grade 2 DIC, other active malignancy within 1 year before study entry, major organ dysfunction, active infections, or pregnancy or breastfeeding.
STATISTICAL CONSIDERATIONS	Standard 3+3 design
TOTAL NUMBER OF SUBJECTS	N = 15-24 (n = 9-18, plus 6).
ESTIMATED ENROLLMENT PERIOD	18 months
ESTIMATED STUDY DURATION	30 months

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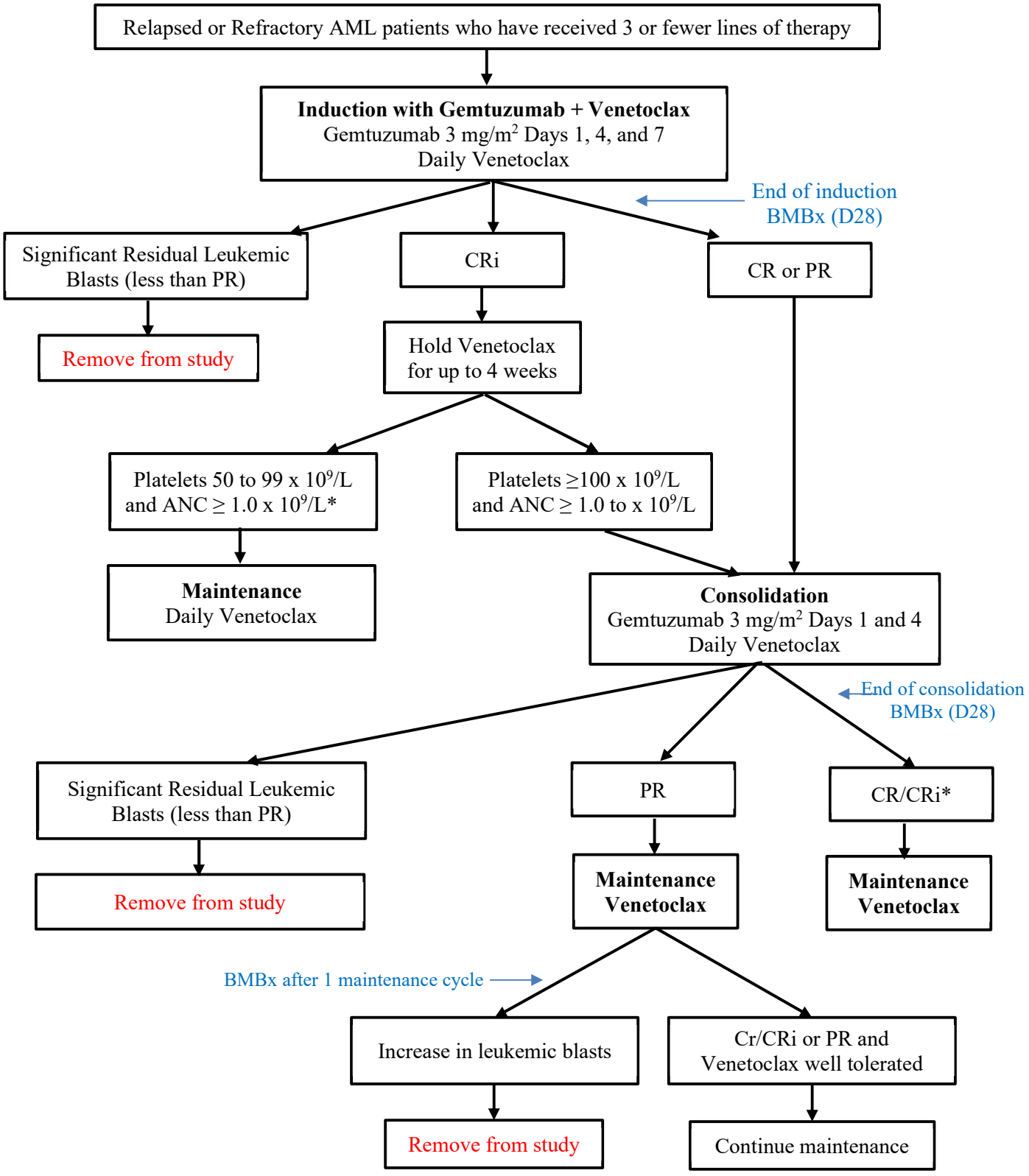
SCHEMA



*If patients have grade 3 thrombocytopenia or neutropenia after holding venetoclax for 4 weeks, remove from study.

ABBREVIATIONS

Abbreviation	Definition
AE	adverse event
AHQ	Administrative Headquarters
ANC	absolute neutrophil count
ALT	alanine aminotransferase
AML	acute myeloid leukemia
aPTT	Activated partial thromboplastin time
AST	aspartate aminotransferase
BCL-2	B-cell lymphoma-2 gene
BH3	BCL-2 homology 3 domain
BM	bone marrow
BMP	basic metabolic panel
BSA	body surface area
BTCRC	Big Ten Cancer Research Consortium
C1D1	Cycle 1 day 1
CBC	complete blood cell count
CI	Confidence interval
CLL	chronic lymphocytic leukemia
CLM	Correlative Laboratory Manual
CNS	Central nervous system
CR	complete remission
CRc	complete cytogenetic response
CRi	complete remission with incomplete hematologic recovery
CRp	complete response with incomplete platelet recovery
CTCAE	(NCI) Common Terminology Criteria for Adverse Events
dL	Deciliter
DLT	dose-limiting toxicity
DSMC	Data Safety Monitoring Committee
DSMP	Data Safety Monitoring Plan
eCRF	electronic case report form
EDC	electronic data capture
ECOG	Eastern Cooperative Oncology Group
EFS	event-free survival

eGFR	Estimated Glomerular Filtration Rate
FDA	Food and Drug Administration
FLT3	FMS-like tyrosine kinase-3
GO	Gemtuzumab Ozogamicin
Hb	Hemoglobin
HBsAg	Hepatitis B surface antigen
HepC	Hepatitis C
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
Hr	Hour
HR	Hazard ratio
HMA	hypomethylating agent
IB	Investigator's Brochure
IHC	Immunohistochemistry
IND	Investigational New Drug
INR	international normalized ratio
IRB	Institutional Review Board
IUD	Intra uterine device
IV	Intravenous
Kg	Kilogram
Lb	Pound
LDAC	low-dose cytarabine
LDH	lactate dehydrogenase
LMWH	low-molecular-weight heparin
LSCs	leukemia stem cells
MCL-1	myeloid cell leukemia-1
Mg	Milligram
Min	Minute
mL	Milliliter
MOMP	mitochondrial outer membrane permeabilization
MRD	minimal residual disease
MTD	maximum tolerated dose
N/A	Not applicable
NCCN	National Comprehensive Cancer Network

NCI	National Cancer Institute
NDC	National Drug Code
NS	Normal saline
ORR	overall response rate
OS	overall survival
PFS	progression-free survival
PK	Pharmacokinetics
PR	partial remission
PT	Prothrombin time
RFS	relapse-free survival
RP2D	Recommend phase 2 dose
RR	response rate
SAE	serious adverse event
SCT	stem cell transplant
SD	stable disease
TLS	tumor lysis syndrome
UI-Health	University of Illinois Health
ULN	upper limit of normal
UPIRSO	unanticipated problems involving risk to subjects or others
US	United States
USP	United States Pharmacopeia
VOD	veno-occlusive disease
WHO	World Health Organization
WOCBP	women of childbearing potential
Wt	Weight

1. BACKGROUND AND RATIONALE

1.1 AML Background

AML is predominantly a disease of the elderly, yet the results of therapy are dismal for these patients, others deemed ineligible for standard cytotoxic induction chemotherapy, and especially for patients with previously treated relapsed/refractory AML. Very few such patients survive more than 2 years.[1] Refractoriness to AML treatment, or relapse, are caused by quiescent leukemia stem cells (LSCs) that are *resistant* to conventional chemotherapy. Targeted AML therapy for such infirm, usually elderly, patients seeks to exploit therapeutic differences between LSCs and normal hematopoietic stem cells to eradicate leukemia, with the aim of providing meaningful survival prolongation, and maintaining or improving their quality of life.

In September 2017, the FDA approved GO for treatment of adult patients with CD33-positive, newly diagnosed or relapsed/refractory, AML. GO's novel mechanism of action in combination with Venetoclax, a drug that has recently proved to be efficacious with manageable toxicities, will be investigated in this study.

1.2 Investigational Treatment

1.2.1 Gemtuzumab Ozogamicin (GO)

GO consists of a humanized anti-CD33 monoclonal antibody linked to calicheamicin, a potent antitumor enediyne antibiotic. GO binds to CD33, an antigen expressed on the surface of more than 90% of AML blast cells. Binding of GO is followed by internalization and intracellular release of calicheamicin, leading to DNA damage and subsequent mitochondria-dependent apoptosis.

The original FDA approval for GO was based on phase II studies of 9 mg/m2 given on days 1 and 14 in 277 patients with AML in first relapse, where it showed good tolerability, an overall response rate (ORR = complete remission (CR) and complete response with incomplete platelet recovery (CRp)) of 26%, and a median relapse-free survival (RFS) of approximately 6 months in patients aged >60 yrs (review[2]). To reduce the hematological and hepatic toxicities seen with this dose and schedule,[3] a French Cooperative group performed a phase II trial of GO 3 mg/m^2 given on days 1, 4, and 7, in patients with relapsed AML (n = 57).[4] The rationale for lower dosing was based on responses observed with doses of 1–4 mg/m2 in the initial phase 1 study of GO. The administration of fractionated doses of GO was derived from observations of rapid re-expression of CD33 on the cell surface after an initial exposure (review[5]). The ORR was 33%, and median survival improved to 11 months, with a marked reduction in adverse effects (e.g., median duration of >grade 2 thrombocytopenia fell to 21 days).

In a randomized phase III Cooperative group trial this GO dosing and schedule was next combined with a standard AML chemotherapy regimen ("7+3") and compared to "7+3" alone—resulting in significant gains in 2 year event-free survival (EFS), overall survival (OS) and RFS, despite no difference in initial CR rates.[6] These benefits were confirmed in a recent meta-analysis as extending to 6 years in AML patients with low or intermediate risk cytogenetics,[7] suggesting addition of GO increases the eradication of LSC, particularly in more "mature" AML with CD33-expressing LSC (reviews[8,9]).

Note that patients in CR or CRp after induction received two further consolidation cycles of Gemtuzumab Ozogamicin (3 mg/m^2 on day 1) according to their initial randomization, in combination with daunorubicin and cytarabine.[6]

There are at least three recognized mechanisms of AML resistance to GO and calicheamicin (reviews[5,9]) including (i) decreased blast CD33 expression, related to common polymorphisms in CD33 splicing;[10,11] (ii) upregulation of drug transporters that re-export calicheamicin (e.g., *p*-glycoprotein[5,12]); and (iii), decreased apoptosis due to deficient activation of mitochondrial outer membrane permeabilization (MOMP), inhibiting the release of cytochrome *c*, subsequent caspase activation and blast cell death.[5,13] Of interest, apoptosis induced by GO (calicheamicin effect) is both CD95/Fas and p53 *independent*, is dependent on the pro-apoptotic proteins Bax/Bak, and strongly inhibited by overexpression of the anti-apoptotic proteins BCL-2 or BCL-XL.[13] Conversely, GO-related apoptosis is increased by drugs that appear to induce MOMP, such as PK11195.[12]

1.2.2 Venetoclax

Recent studies indicate that LSCs have a unique metabolic phenotype, with low levels of reactive oxygen species, a reliance on mitochondrial oxidative phosphorylation (rather than aerobic glycolysis), and aberrant overexpression of the B-cell lymphoma-2 gene (BCL-2). Inhibition of BCL-2 selectively targets mitochondrial energy metabolism in LSCs, and, due to their unique inability to upregulate glycolysis for adenosine triphosphate (ATP) production, induces cell death in LSCs but not in hematopoietic stem cells.[14]

Venetoclax (ABT-199) is a recently developed BCL-2 homology 3 domain (BH3) mimetic, binding BCL-2, and dislodging it from binding to Bak and Bax, to allow apoptosis.[15] Thus it should specifically impact LSCs. Venetoclax is orally bioavailable, has demonstrated potent anti-leukemic activity in AML cell lines and primary patient samples, including LSCs, and is now being intensively studied in various hematologic malignancies, including AML.[16,17] Notably, this BH3 mimetic has been granted FDA approval for treatment of previously treated chronic lymphocytic leukemia (CLL[18]) patients with 17p deletion after a phase II study showed an overall response rate of nearly 80%.

As with most AML therapies, use of BH3 mimetics as monotherapy for AML is unlikely to be curative: acquired ABT-199 resistance rapidly occurs due to, for example, upregulation of other anti-apoptotic proteins such as BCL-XL or myeloid cell leukemia-1 (MCL-1), and their inhibition is then required to prevent glycolysis and induce LSC death in vitro.[19] In accord with these observations, in *in vivo* studies, a recent phase II trial of ABT-199 monotherapy resulted in a CR rate of 19% in previously treated relapsed/refractory ("high risk") AML patients, but median time to relapse was only 75 days.[20] Thus, unlike in CLL, where BCL-2 is of primary importance, there is heterogeneous use of antiapoptotic proteins by AML LSCs, and *more broad-based targeting using a LSC-targeting BH3 mimetic in combination with chemotherapies known to target LSCs (such as GO) may be more efficacious*.[15,20]

To date, early phase trials have demonstrated that in AML patients ineligible for standard "7+3" induction therapy, the addition of Venetoclax to standard "less intensive therapies" improves response rates while maintaining a tolerable safety profile. In an ongoing phase II study of Venetoclax (400 or 800 mg) in combination with standard dosing of the hypomethylating agents (HMA) Decitabine or Azacitidine in de novo AML patients, the ORR was over 70%. This lead to FDA breakthrough designation in January 2016 for "Venetoclax use in combination with HMAs in treatment-naive patients ineligible for standard therapy".[21] In another early phase study Lin et al. combined Venetoclax with low-dose cytarabine (LDAC) and observed improved overall survival rates when compared with single agent LDAC.[22] In these reported studies (n = 74) the most common grade 3/4 adverse events were those typically seen in AML-febrile

neutropenia, hypokalemia, and pneumonia.[20-22] Of note, no reports suggested increased risk of hepatotoxicity or thrombocytopenia.

Of interest, patients destined to respond to ABT-199 monotherapy in the phase II trial above[20] were retrospectively identified based on "BH3 profiling" of their pretreatment AML blasts. BH3 profiling is a well-published assay to determine mitochondrial sensitivity to BH3 peptides, reflecting cancer cell "priming" or dependency on BCL-2 (in this case), BCL-XL, and/or MCL-1 for apoptosis protection. Importantly, BH3 profiling also strongly predicts LSC chemosensitivity.[20,23]

1.3 Rationale

Based on previous studies, we propose a phase Ib dose-finding study of Venetoclax in combination with staggered dosing of GO for refractory/relapsed CD33+ AML patients, as a combination therapy that has several important synergies.

1. By targeting BCL-2 proteins that protect AML cells from apoptosis, Venetoclax should increase the efficacy of GO, overcoming the resistance of leukemic cells to calicheamicin.
2. They should have synergistic effects in eradicating LSCs (although they may target separate LSC populations).
3. The combination should potentially target both low-, intermediate-, and high-risk AML patients (this population).
4. Staggered dosing of GO may compensate for the low expression of CD33/slow uptake of GO in some patients with AML.[12]

Most importantly, we believe this combination can provide meaningful survival prolongation in this vulnerable population, while maintaining or improving their quality of life.

1.3.1 Rationale for enrolling patients into cohort 3 who are resistant to venetoclax

Since the combination of venetoclax and hypomethylating agents (HMA) was granted accelerated approved by the FDA in November of 2018,[24] its use in the front-line setting – as well as its off-label use in the relapsed/refractory AML (R/R AML) setting – has increased.

However, there is a rationale for using venetoclax with gemtuzumab ozogamicin (GO) in venetoclax-refractory patients. GO induces anti-leukemic activity by causing DNA scission in leukemic cells, which activates an apoptotic pathway that results in mitochondrial outer membrane permeabilization (MOMP).[25] In many AML samples BCL-2 overexpression occurs, decreasing MOMP.[13] The rationale for use of venetoclax in BTCRC AML17-113 is to counteract this mechanism of apoptotic resistance.

It is not clear how AML cells become resistant to venetoclax. One significant mechanism of resistance in CLL cells is that they undergo mutations in the BCL-2 gene which reduce binding by venetoclax.[26] However whether this particular mechanism of resistance occurs in AML is unknown. Studies on small numbers of patients receiving venetoclax-based combinations in AML have been performed. One such study looked at 25 patients, out of a total of 81 treated patients, who experienced adaptive resistance to venetoclax and HMA or low dose cytarabine. Of these 25 patients, 5 developed an increase in FLT3-ITD mutational burden, while 15 developed TP53 mutations/deletions.[27] Notably, GO is known to have reduced activity in the context of TP53 mutations (TP53 upregulates the pro-apoptotic proteins NOXA, PUMA, BAX, BIM), whereas FLT3-ITD mutations do not have a clinically meaningful effect on GO efficacy.[28]

Thus while BCL-2:venetoclax interactions may still be occurring in venetoclax-resistant AML cells, it appears other mechanisms are likely preventing these cells from undergoing apoptosis, for example, upregulation of MCL-1 or BCL-XL.[29] One may (simplistically) model intrinsic apoptosis as being prevented by the summation of these 3 important proteins, MCL-1, BCL-XL and BCL-2, thus the binding of BCL-2 by venetoclax should still aid apoptotic stimuli in that it reduces the total amount of anti-apoptotic proteins in venetoclax-resistant cells. As an example of this the downregulation of MCL-1 by omacetaxine has been shown to overcome venetoclax resistance, resensitizing leukemia cells to *BCL-2 inhibition by venetoclax*. In addition, calicheamicin is a potent driver of apoptosis, in contrast to HMA, which, at the doses used in the venetoclax/HMA regimen, are not directly cytotoxic.[30] Thus, by using Gemtuzamab to increase the apoptotic stimulus, requiring increased levels of anti-apoptotic proteins, previously venetoclax-resistant leukemia cells should be induced to undergo apoptosis.

In sum, we hypothesize that recruiting patients into BTCRC AML17-113 cohort 3 who are resistant to venetoclax is a therapeutically rational choice for the following reasons:

(1) This cohort uses a dose of venetoclax, 600 mg, higher than the dose given with HMA (400 mg). Greater downregulation of BCL-2 should expose AML cells to greater apoptotic pressure.
(2) GO should provide a greater stimulus to apoptosis, and the continued use of venetoclax means GO should provide this stimulus without being inhibited by BCL-2.
(3) As TP53 mutations or deletions decrease the efficacy of GO, patients with AML who have been exposed to venetoclax *AND* who have developed TP53 mutations/deletion are likely not good candidates for this trial.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 Objectives

2.1.1 Primary Objective

- Assess the maximum tolerated dose (MTD) of Venetoclax when administered with GO in patients with AML.

2.1.2 Secondary Objectives

- Estimate the efficacy of the combination in achieving ORR after first two cycles (overall response rate includes CR/CRi).
- Estimate the efficacy of the combination in achieving anti-leukemic activity (CR/CRi/PR).
- Characterize any adverse effects of GO in combination with Venetoclax.
- Estimate the relapse-free, event-free and overall survival.
- Longitudinal quantitative assessment of the effects of therapy on quality of life (QOL), degree of fatigue, and physical function.

2.1.3 Exploratory Objectives

- Longitudinal assessment of genomic minimal residual disease (MRD) to determine the kinetics of response and the association with relapse-free survival.
- Explore the impact of CD33 variants, and the degree of CD33 expression on leukemic blasts, on the response rates and relapse-free survival.
- Explore the relationship between leukemic blast BH3 profiling and response rates.

- Explore the relationship between leukemic blast expression of anti-apoptosis proteins (BCL-2, BCL-XL and MCL-1) and response rates.

2.2 Endpoints

2.2.1 Primary Endpoint

- Dose-limiting toxicity, defined as an adverse event related (possible, probably, or definite) to Venetoclax and/or Gemtuzumab fulfilling one of the following criteria:
  - Hematologic toxicity: treatment-related grade 3 or worse neutropenia and/or thrombocytopenia due to bone marrow hypocellularity present at the end of cycle one (day 28) with an additional 28 days allowed for count recovery (i.e. present at day 56); specifically grade 3 or worse neutropenia or thrombocytopenia with the bone marrow documented to be free of leukemic infiltration. Note: patients who enter the study with grade 3 or worse cytopenias will not be evaluable for hematologic dose-limiting toxicities.
  - Non-hematologic toxicity: any grade 3 or worse treatment-related toxicity occurring within the first cycle (excluding grade 3-4 infections during cycle one).

2.2.2 Secondary Endpoints

- Overall response rate (CR/CRi), as defined by the revised IWG criteria[31]. See Section 9.1.
- Anti-leukemic activity (CR/CRi/PR), as defined by the revised IWG criteria[31]. See Section 9.1.
- Adverse effects will be characterized using the CTCAE v5 criteria.
- Relapse-free survival (RFS), event-free survival (EFS, and overall survival (OS). See Section 9.2
- The European Organization for the Research and Treatment of Cancer 30 item questionnaire (EORTC QLQ-C30), The Functional Assessment of Cancer Therapy Fatigue (FACT-Fatigue) questionnaire, two-minute walk test, and timed chair stands will be performed at trial entry and every other month for 1 year.[32]

2.2.3 Correlative/ Exploratory Endpoints

- Genomic MRD with droplet digital PCR (ddPCR) will be measured to determine kinetics of response and the association with relapse-free survival at the end of cycles 1 and 2, at 6 months and 12 months, and if there is evidence of relapse. (Sub-PI is Dr. Brian Parkin [see ref. 33].)
- CD33 DNA polymorphisms will be measured at presentation and at relapse.
- Leukemic blast CD33 expression will be measured at presentation and at relapse.
- "BH3 profiling"[34] of AML blasts will be performed at presentation and at relapse.
- BCL-2, BCL-XL, and MCL-1 protein levels in PB or BM blasts will be measured at presentation and after 6 days (i.e., Cycle 1 Day +3) of Venetoclax therapy.

3. ELIGIBILITY CRITERIA

Study entry is open to adults regardless of gender or ethnic background. While there will be every effort to seek out and include women and minorities, the patient population is expected to be no different than that of other cancer studies at the hospitals participating in this trial.

3.1 Inclusion Criteria

Subjects 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. Ages 18 to 75 years at the time of consent.

3. ECOG Performance Status of 0-2 within 7 days prior to registration; see Appendix I.

4. Patients must have AML, as defined,[35] that is relapsed or refractory. Prior therapy including chemotherapy, immunotherapy, biological or targeted therapy (e.g. FMS-like tyrosine kinase-3 (FLT3) inhibitors, other kinase inhibitors, azacitidine, ATRA) is allowed.

5. CD33 expression (by flow or IHC) in at least 20% of the leukemia blasts per local pathologist.[36]

6. Prior cancer treatment must be completed at least 21 days prior to registration and the subject must have recovered from all reversible acute toxic effects of the regimen (other than alopecia) to ≤Grade 1 or baseline.

7. 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	≥30 cc/min using the Cockcroft-Gault formula
Hepatic	
Bilirubin	≤1.5 × upper limit of normal (ULN)
Aspartate aminotransferase (AST)	≤2 × ULN
Alanine aminotransferase (ALT)	≤2 ×ULN

8. Females of childbearing potential must have a negative serum or urine pregnancy test within 7 days prior to registration. **NOTE:** Females are considered of child bearing potential unless they are surgically sterile (have undergone a hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or they are naturally postmenopausal for at least 12 consecutive months

9. Females of childbearing potential and males must be willing to use effective contraception during treatment and for at least 30 days after the last dose of Venetoclax. Females will be advised to use effective contraception for at least 6 months after the last dose of Gemtuzumab and males for at least 3 months after the last dose of Gemtuzumab.

10. As determined by the enrolling physician or protocol designee, ability of the subject to understand and comply with study procedures for the entire length of the study

3.2 Exclusion Criteria

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

1. Patients with history of prior use of GO or Venetoclax
   NOTE: Starting with dose cohort 3, prior therapy with venetoclax is allowed, provided patients do not have evidence of p53 deletion or mutations. If the dose cohort is de-escalated to dose cohort 2 due to toxicity in cohort 3, prior exposure to venetoclax will continue to be allowed, provided patients do not have evidence of p53 deletion/mutations.

2. History of myeloproliferative neoplasm [MPN] including myelofibrosis, essential thrombocythemia, polycythemia vera, CML with or without BCR-ABL1 translocation, and AML with BCR-ABL1 translocation.

3. More than three lines of prior therapy. A line of therapy consists of ≥1 complete cycle of a single agent, a regimen consisting of a combination of several drugs, or a planned sequential therapy of various regimens (e.g., 3-6 cycles of initial therapy with bortezomib-dexamethasone [VD] followed by stem cell transplantation [SCT], consolidation, and lenalidomide maintenance is considered 1 line).[37]

4. WBC >25 × 10^9/L. Cytoreduction is required (hydroxyurea as per local standard of care).

5. Acute promyelocytic leukemia.

6. Unresolved ≥grade 2 clinically significant nonhematologic toxicities from prior anticancer therapy or *unresolved* disseminated intravascular coagulation ≥ grade 2 per CTCAE v5 criteria.

7. History of other malignancies within 1 year prior to study entry, with the exception of: adequately treated *in situ* carcinoma of the cervix uteri or carcinoma *in situ* of breast; basal cell carcinoma of the skin or localized squamous cell carcinoma of the skin; previous malignancy confined and surgically resected (or treated with other modalities), with curative intent.

8. Investigational drug within 4 weeks of study entry.

9. History of CHF requiring treatment, left ventricular ejection fraction ≤ 50%, cardiac insufficiency grade III or IV per New York Heart Association classification (NYHA; see Appendix II), or chronic stable angina

10. Patients who are HIV positive.

11. Known CNS involvement with AML.

12. Previous hematopoietic stem cell transplant within 2 months.

13. Previous history of veno-occlusive disease/sinusoidal obstruction syndrome.

14. Patients who are positive for hepatitis B or C infection with the exception of those with an undetectable viral load within 3 months. Subjects with serologic evidence of prior vaccination to HBV [i.e., HBs Ag-, and anti-HBs+] may participate.

15. Active uncontrolled infection or severe systemic infection. Enrollment is possible after control of infection, at discretion of the treating physician.

16. Pregnant or breastfeeding (**NOTE:** breast milk cannot be stored for future use while the mother is being treated on study).

17. Patients who have received strong and/or moderate CYP3A inducers or inhibitors within 7 days prior to the initiation of study treatment unless deemed necessary by the treating physician. (See Section 5.8.2 and Appendix III)

18. Patients who have consumed grapefruit, grapefruit products, Seville oranges (including marmalade containing Seville oranges) or Starfruit within 3 days prior to the initiation of study treatment.

19. Malabsorption syndrome or other condition that *precludes* enteral route of administration.

20. Psychological, familial, sociological, or geographical condition that would preclude study compliance and follow-up.

21. Unable or unwilling to undergo a screening bone marrow study.

22. Other severe acute or chronic medical or psychiatric condition, or laboratory abnormality that may increase the risk associated with study participation or investigational product administration or may interfere with the interpretation of study results and, in the judgment of the investigator, would make the patient inappropriate for enrollment in this study.

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. A subject is considered registered when an 'On Study' date is entered into the EDC system.

Registration will occur after the patient has signed the patient consent and eligibility is confirmed, but before any treatment has been administered. To be eligible for registration to this study, the patient must meet each of the criteria listed on the eligibility checklist. A copy of the eligibility checklist will be retained at the participating center. Subjects must begin therapy within **5 business days** of registration.

Venetoclax dose levels will be assigned at registration.

If a patient signs consent and is registered to the study and is later found to be unable to begin the planned study treatment, for whatever reason, the patient will be removed from study and treated at the physician's discretion. The patient will be considered a screen/baseline failure and be replaced. Further data will not be collected if the patient has not begun study treatment at the time of removal from trial. The reason for removal from study will be clearly indicated in EDC.
If a patient begins treatment, and then treatment is discontinued for whatever reason, the patient must be followed per section 7.2.

5. TREATMENT PLAN

This will be a single arm, open-label multicenter study of the combination of Venetoclax and GO in patients with AML. It will be an initial dose-escalation phase Ib study (Cohorts 1-3); using a standard 3+3 design to determine the maximum tolerated dose. There will be an induction and a consolidation cycle followed by a maintenance phase.

5.1 Dose Escalation Rules

- Three to six subjects will initially be enrolled at dose level 1. If none of the 3 subjects experience a dose limiting toxicity (DLT) during the first cycle of therapy, an additional three subjects will be enrolled at dose level 2. If none of the 3 subjects at dose level 2 experience a dose limiting toxicity (DLT), an additional three subjects will be enrolled at dose level 3. If all subjects in dose level 3 complete the first cycle of therapy without DLT, 3 more subjects will be enrolled into dose level 3 to ensure only 0-1 of 6 subjects have a DLT. There will be no further escalation beyond dose level 3.

- Alternatively, if 1 of the first 3 subjects within any given dose cohort experiences a 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 a 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 a 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. If this occurs in cohort 1, the dose will be decreased to cohort -1 and if 2 or more DLTs are reached in dose level -1, the study will be put on hold and a protocol amendment will be considered. [see Section 6.4 for "Stopping Rules"]

- The MTD for Venetoclax in combination with Gemtuzumab Ozogamicin 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. That dose will be the recommended Phase II dose (RP2D).

- Once the RP2D has been identified, we will expand this cohort to include an additional six patients to further evaluate the efficacy and safety profile of the RP2D.

5.2 Definition of Dose-limiting Toxicities

Dose-limiting toxicities (DLTs) are defined as an adverse event related (possible, probable or definite) to Venetoclax and/or Gemtuzumab Ozogamicin fulfilling one of the following criteria:

- Hematologic toxicity: treatment-related grade 3 or worse neutropenia and/or thrombocytopenia due to bone marrow hypocellularity present at the end of cycle one (day 28) with an additional 28 days allowed for count recovery (i.e. present at day 56);

specifically grade 3 or worse neutropenia or thrombocytopenia with the bone marrow documented to be free of leukemic infiltration. Note: patients who enter the study with grade 3 or worse cytopenias will not be evaluable for hematologic dose-limiting toxicities.

- Non-hematologic toxicity: any grade 3 or worse treatment-related toxicity occurring within the first cycle (excluding grade 3-4 infections during cycle one).

NOTE: Events that can be clearly attributed to disease progression, concurrent illness or the effects of other medications will not be classified as DLTs.

DLTs will be counted based on the number of patients with a DLT at a given dose level, not the absolute number of DLTs. No single patient can trigger more than one DLT. Intra-patient dose escalation is not permitted.

5.3 Dose Cohorts

Cohort	Number of Subjects	Venetoclax Daily Dose (mg)	Gemtuzumab Ozogamicin IV on Days +1, +4, +7
–1	3-6	100	3 mg/m^2 (up to one 4.5 mg vial)
1 (start)	3-6	200	
2	3-6	400	
3	3-6	600	

5.4 Induction (Cycle 1) and Consolidation (Cycle 2):

Cycle 1: Gemtuzumab Ozogamicin (GO) 3 mg/m^2 IV infusion (up to one 4.5 mg vial) will be given on days +1, +4, and +7 in combination with daily oral administration of Venetoclax during Induction. Based on previous studies,[21,22] Venetoclax dose will start at 200 mg daily with dose escalation planned over 3 cohorts to identify the recommended phase 2 dose (RP2D). Dose cohort 3 will use the maximum dose of Venetoclax of 600 mg orally daily. Because of evidence of tumor lysis syndrome (TLS) with Venetoclax use in previous CLL studies,[18] we will use a 3-day stepwise ramp up in cycle 1 to the target dose for all cohorts (see Table 1).[20-22] Patients should expect to be hospitalized throughout Induction/Cycle 1, although medically stable patients may be discharged from the hospital under close supervision at the discretion of the treating physician. Patients do not have to be hospitalized during the first week of consolidation if the treating physician wishes to administer Gemtuzumab (C2D1 and D4) in the outpatient clinic.

Table 1. Venetoclax Ramp-up schedule

Day	Cohort 1 (mg)	Cohort 2 (mg)	Cohort 3 (mg)
–2	50	50	100
–1	100	100	200
0	100	200	400
1–28	200	400	600

Clinical and hematological responses will be assessed after the end of both the induction and consolidation cycles. Bone marrow assessment will occur on day 28 regardless of absolute neutrophil count (ANC) or platelet recovery.

After induction, patients demonstrating complete remission or partial remission will continue to consolidation. Patients in CRi (ANC < 1.0×10^9/L or platelets < 100×10^9/L) will stop Venetoclax for up to four weeks to allow for cytopenia recovery. If the ANC recovers to ≥ 1.0×10^9/L and the platelets recover to ≥100×10^9/L, consolidation will ensue. If there is not full ANC AND platelet recovery to the aforementioned levels, consolidation will be omitted, and patients will enter the maintenance phase with Venetoclax alone provided they do not have any grade 3-4 cytopenias. The previously assigned Venetoclax dose will be resumed and the maintenance rules for Venetoclax dose adjustments or discontinuation will apply (see section **6.2.2**). If however patients have a grade 3-4 cytopenia despite holding Venetoclax for four weeks, they will be *removed from study* [**NOTE**: this applies to both induction and consolidation.]

Cycle 2: The second cycle (consolidation) will consist of GO 3 mg/m^2 IV infusion (up to one 4.5 mg vial) on days +1, and +4, in addition to daily oral administration of Venetoclax at the assigned dose. A bone marrow assessment will occur on day 28 regardless of cytopenias and those with CR, CRi, or at least a partial remission will continue onto maintenance therapy with Venetoclax alone. The previously assigned Venetoclax dose will be resumed and the maintenance rules for Venetoclax dose adjustments or discontinuation will apply (see section **6.2.2**).

After day 28, in the absence of leukemia in the BM, where there is a grade 3-4 cytopenia (thrombocytopenia or neutropenia), Venetoclax should be held for up to four weeks to allow recovery of counts to platelets ≥ 50 x 10^9/L and ANC ≥1.0 x 10^9/L (to allow entry into Maintenance Phase). Those who continue to have a grade 3-4 cytopenia will be taken off study.

Patients with treatment failure (see Section 9.1) will be taken off study. Please note that any patient with residual disease (with at least PR) may move onto consolidation regardless of blood counts.

5.5 Maintenance Phase: *(see also section 6.2.2, Tables 7, 8)*

Please note that any patient with residual disease (with at least PR) may move onto maintenance regardless of blood counts.

Patients who enter a maintenance phase require platelets ≥ 50 x 10^9/L and an ANC ≥ 1.0×10^9/L. If a subject presents with a Grade 4 cytopenia for more than 1 week during Maintenance phase, unless it is thought to be due to the underlying disease, venetoclax dosing should be interrupted until platelets ≥ 50 x 10^9/L and ANC ≥ 1.0 x 10^9/L.

During Maintenance, for subjects, in CR/CRi, receiving venetoclax who required >1 interruption or delay of study drug administration for grade 4 cytopenias, venetoclax, at dose prescribed for that cohort, should be administered for 21 days out of 28 days during each of the subsequent cycles. If, despite these modifications, a subject continues to have persistent grade 4 cytopenias, venetoclax should be administered for 14 days out of 28 days during each of the subsequent cycles.

If grade 3 cytopenias (i.e., ANC < 1.0 x 10^9/L and/or platelets < 50 x 10^9/L) persist please discuss with PI, however those who still continue to have a grade 4 cytopenia should be taken off study.

Venetoclax therapy will be continued daily at the previously assigned dose for as long as patients continue to benefit or until disease progression or unacceptable toxicity.

Patients who enter the maintenance phase in partial remission should have a bone marrow assessment after the first month of maintenance therapy (cycle 3). Those with an improved response should continue with maintenance Venetoclax. Those with stable disease should continue treatment if the drug is well-tolerated and if the primary hematologist believes that Venetoclax is of benefit. If there is disease progression, patients should be taken off study.

5.6 Transplant-eligible Patients

Patients deemed suitable for transplant may be removed from the study at any point, at the discretion of the treating physician. We suggest a period of 2-3 months between the last dose of Gemtuzumab and the beginning of any HSCT conditioning regimen. In view of the infrequent association of VOD with Gemtuzumab use, we recommend the consideration of alternatives to TBI/TMI-containing protocols, which may increase the risk of VOD, for these patients. Ultimately, the final decision is deferred to the site PI.

5.7 Pre-medication and Hydration

Gemtuzumab:

Premedicate with acetaminophen 650 mg orally and diphenhydramine 50 mg orally or intravenously one hour (±15 min) prior to the administration and methylprednisolone 1 mg/kg or an equivalent dose of an alternative corticosteroid within 30 minutes (±10 min) prior to the infusion of GO.

Additional doses of acetaminophen and diphenhydramine may be administered every 4 hours after the initial pretreatment dose. It is advised to repeat the same dose of methylprednisolone or an equivalent corticosteroid for any sign of an infusion reaction (e.g., fever, chills, hypotension or dyspnea) during the infusion or within 4 hours after the initial pretreatment dose.

NOTE: For patients with hyperleukocytosis (leukocyte count greater than 25 x 10^9/L), cytoreduction (hydroxyurea) is required prior to starting treatment.

Venetoclax:

Venetoclax can cause rapid reduction in tumor thereby increasing the risk for TLS, despite the initial ramp-up phase. To mitigate risk of TLS, patients should receive 1.5 – 2 L hydration (oral or IV) daily in addition to use of a uric acid-lower agent starting at D-2 and continued until physician discretion.

Per the National Comprehensive Cancer Network (NCCN) guideline, Venetoclax is classified as having a minimal to low emetic risk (<30% frequency of emesis). Antiemetic medications for breakthrough nausea will be prescribed at the primary investigator's discretion.

If a patient misses a dose of Venetoclax within 8 hours of the scheduled dose, the patient should take the missed dose and then resume the normal daily dosing schedule. If a dose is missed by more than 8 hours, the patient should not take the missed dose and resume the daily dosing schedule the following day. If a patient vomits after a dose, no additional dose should be taken.

NOTE: For patients with hyperleukocytosis (leukocyte count greater than 25 x 10^9/L), cytoreduction (hydroxyurea) is required prior to starting treatment.

Table 2. Induction (Cycle 1) and Consolidation (Cycle 2) Venetoclax and Gemtuzumab Administration

Drug	Dose[1]	Route	Schedule	Cycle Length
Venetoclax[3]	Assigned dose[2]	Orally	Days 1-28 (Cycle 1: Day -2, -1, 0)[2]	28 days
Gemtuzumab	3 mg/m^2 (up to one 4.5 mg vial)	Infusion	**Cycle 1**: Days 1, 4, and 7	
			Cycle 2: Days 1, 4	

[1]Body surface area (BSA) should be recalculated when weight changes by ≥ 10% according to the Mosteller formula.
[2]Please see Table 1 stepwise ramp-up schema
[3]Venetoclax tablets should be taken orally once daily with a meal and water. Do not chew, crush or break tablets. **NOTE:** Diaries will be provided for self-administration of Venetoclax when subjects are outpatient. On days when both venetoclax and gemtuzumab are given, it is preferred that subjects take venetoclax 1 hour prior to the GO infusion. However, variations from this guidance will not be considered as protocol deviations.

Table 3. Maintenance Venetoclax Administration (Cycles 3+)

Drug	Dose	Route	Schedule	Cycle Length
Venetoclax[1]	Assigned dose	Orally	Days 1-28	28 days

[1]Venetoclax tablets should be taken orally once daily with a meal and water. Do not chew, crush or break tablets. **NOTE:** Diaries will be provided for self-administration of Venetoclax when subjects are outpatient.

5.8 Concomitant Medications

5.8.1 Allowed Concomitant Medications

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

Appropriate use of transfusions is recommended. Please refer to Supportive Care section for further details.

Due to potential interference of azole antifungals with Venetoclax metabolism, non-azole

antifungals are recommended if antifungal prophylactic medications are used during prolonged episodes of grade 4 neutropenia (Induction phase).

5.8.2 Prohibited Concomitant Medications

Please see Appendix III for a table of prohibited and cautionary medications.

In general, hematopoietic growth factors should not be used unless deemed to be necessary by the treating physician (see also section **5.7**).

Strong CYP3A Inhibitors

Venetoclax is predominantly metabolized by CYP3A4/5 therefore concomitant use with strong CYP3A inhibitors (e.g., ketoconazole, conivaptan, clarithromycin, indinavir, itraconazole, lopinavir, ritonavir, ritonavir-boosted nirmatrelvir [Paxlovid], telaprevir, posaconazole, voriconazole) at initiation should be avoided. If use is deemed medically necessary by the treating physician (recognizing that this may increase the risk of tumor lysis syndrome), see Table 4 for dosing instructions. For patients who have completed the ramp-up phase and are on a steady daily dose of Venetoclax, see Table 4 for steady dose instructions[38,39] – note that Posaconazole requires greater dose reductions than other strong CYP3A inhibitors. If the strong CYP3A inhibitor is discontinued, resume the Venetoclax dose that was used prior to initiating the CYP3A inhibitor 2 to 3 days after discontinuation.[39]

Table 4: Dose adjustments of Venetoclax in presence of Posaconazole or other strong CYP3A inhibitors

Cohort	Assigned VEN Daily Dose (mg)	Ramp-up day	VEN dose with Posaconazole Daily Dose (mg)	VEN dose with all other strong CYP3A inhibitors Daily Dose (mg)
-1	100	D-2	10	10
		D-1	10	10
		D0	10	20
		Steady dose	20	30
1	200	D-2	10	10
		D-1	20	20
		D0	30	30
		Steady dose	40	50
2	400	D-2	10	10
		D-1	20	20
		D0	50	50
		Steady dose	70	100
3	600	D-2	20	20
		D-1	40	50
		D0	70	100
		Steady dose	100	150

Moderate CYP3A inhibitors and P-gp substrates
Avoid use of moderate CYP3A inhibitors (e.g., erythromycin, ciprofloxacin (*not levofloxacin*), diltiazem, dronedarone, fluconazole *(>200mg),* verapamil). Venetoclax should be administered using caution with substrates or inhibitors of P-gp. Studies indicate that venetoclax may increase the concentration of P-gp substrates. Narrow therapeutic substrates should be administered six hours prior to venetoclax administration.[40] If concurrent use cannot be avoided, reduce the Venetoclax dose by at least 50%. Resume the Venetoclax dose that was used prior to initiating the CYP3A inhibitor 2 to 3 days after discontinuation of the inhibitor.

CYP3A Inducers
Avoid use of strong CYP3A inducers (e.g., carbamazepine, phenytoin, rifampin, St. John's wort) or moderate CYP3A inducers (e.g., bosentan, efavirenz, etravirine, modafinil, nafcillin).

5.9 Supportive Care

Monitoring for tumor lysis is recommended and aggressive hydration and allopurinol prophylaxis should be implemented (see section **5.6**.)

It is recommended that the patient's fluid status and hepatic and renal function and blood glucose levels be carefully monitored during the drug administration period.

Venous Access: A central venous access device is strongly recommended for this study.

Blood Product Support: Appropriate use of all blood products is recommended. Use of cytomegalovirus (CMV) negative products is strongly encouraged for patients documented to be CMV negative and who may later undergo bone marrow transplantation.

Blood products should be irradiated following the current FDA guidelines found at: http://www.fda.gov/cber/gdlns/gamma.html

Hematopoietic Growth Factors: Filgrastim (G-CSF) may be used at the treating physician's discretion, provided bone marrow evaluation demonstrates no evidence of AML, to enhance neutrophil recovery when clinically indicated. The routine use of filgrastim in clinically well patients awaiting count recovery is not recommended.

Prevention of Fungal Infections: Antifungal prophylaxis can reduce morbidity and fungal infection-related mortality in severely neutropenic chemotherapy recipients. Evidence for benefit is strongest for those conditions associated with > 15% rate of systemic fungal infection such as prolonged neutropenia, as observed in AML patients and stem cell transplant (SCT) recipients and is recommended for study patients during Induction. Hospitalized patients who require prophylaxis should receive micafungin, or other non-azole agents (see section **5.8** above).

Management of Fever and Neutropenia: Patients with an ANC < 500/µL (or < 1000/µL and falling) and an oral temperature > 38°C twice in 24 hours or > 38.3°C once, should have empiric systemic antibiotics initiated immediately after appropriate cultures are drawn. The specific choice of empiric antibiotics should be guided by the resistance patterns seen at the individual institution.
The antibiotic regimen chosen for febrile neutropenia should contain activity against gram-

negative organisms and *Pseudomonas aeruginosa* in particular. Note: aminoglycosides increase the risk of nephrotoxicity.

The persistence of fever for > 3 days despite broad spectrum antibiotic therapy or the emergence of a new fever in a neutropenic patient warrants investigation for invasive fungal infection and initiation of empiric antifungal therapy. Antifungal options include echinocandins (micafungin, capsofungin, and anidulafungin), amphotericin B (or lipid amphotericin products), as well as combination therapy. Azole antifungal medications should be avoided, if possible. The choice of specific agent(s) and length of therapy will be dictated by the suspected or confirmed fungal species, site of infection, and clinical status. Consultation with an Infectious Disease physician is recommended.

6. TOXICITIES AND DOSE DELAYS/DOSE MODIFICATIONS

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.

The intent of the study design is for all patients to complete induction, consolidation and maintenance with the exception of those who experience an unacceptable toxicity or if the investigator determines that discontinuation of treatment is in the best interest of the patient. Please see sections **6.2.1** and **6.2.2** below for individual drug exceptions that are applicable throughout the entire study.

6.1 Toxicity

The NCI Common Terminology Criteria for Adverse Events (CTCAE) v5 will be used to grade adverse events. DLTs are defined in detail in section 5.2

6.1.1 Hematologic Toxicity

DLT definition: Grade 3-4 or worse neutropenia or thrombocytopenia occurring after day 56 due to bone marrow hypocellularity and with the bone marrow documented to be free of leukemic infiltration.

6.1.2 Non-hematologic Toxicity

DLT definition: Defined as any grade 3 or worse treatment-related toxicity (excluding grade 4 infections) occurring within the first 56 days.

In general, dose interruptions for grade 3 or 4 non-hematologic toxicities occurring after day 56 will be at the discretion of the primary oncologist. Please see the individual drug sections below for further detail on dose modifications and special exceptions [tables 5,6, 8].

6.2 Dose delays/modifications

During induction and consolidation, there will be no dose reductions of study drugs for hematologic toxicity. For patients in CRi after day 28 of induction, Venetoclax may be held for up to four weeks, to allow for platelet or ANC recovery. *Note CBC will be performed at least twice weekly and consolidation should start as soon as platelets recover to* 100 x 10^9/L *and ANC recovers to* 1.0 x 10^9/L. If platelets or ANC do not recover within 4 weeks, patients will not receive further GO and will enter the Venetoclax maintenance phase. Patients who enter the maintenance phase must have platelets ≥ 50 x 10^9/L and an ANC ≥ 1.0 × 10^9/L. If there is a grade 3 or worse cytopenia present after holding Venetoclax for up to 4 weeks, patients will be removed from study.

At the end of consolidation, Venetoclax may be held again for up to four weeks, maintenance phase can be started as soon thrombocytopenia or neutropenia improves to less than a grade 3 toxicity, unless there is still residual disease in the bone marrow, in which case treatment can proceed regardless of ANC/platelet count. NOTE: If a grade 3 cytopenia persists after 4 weeks of holding Venetoclax for up to 4 weeks following induction or consolidation, patients will be removed from study.

6.2.1 Gemtuzumab

Table 5. Gemtuzumab Delay/Discontinuation

Event	Gemtuzumab Action
Persistent Thrombocytopenia	Discontinue if platelets do not recover to ≥ 100 x 10^9/L after temporary cessation of Venetoclax for four weeks
Persistent neutropenia	Discontinue if ANC does not recover to ≥ 1.0 x 10^9/L after temporary cessation of Venetoclax for four weeks
VOD	Discontinue
Total bilirubin > 2 × ULN, or AST and/or ALT > 2.5 × ULN	Interrupt until recovery of total bilirubin to ≤2 times ULN and AST and/or ALT to ≤2.5 times ULN.

The scheduled dose will be omitted if there is a delay of 3-6 days between sequential infusions.

Table 6. Gemtuzumab Schedule for Interruptions

Time from missed dose	Action
<3 days	Give missed dose
3-6 days	Skip dose
≥7 days	Off study

6.2.2 Venetoclax[39, 41]

Table 7. Dose Duration Reduction for Venetoclax Toxicity (Maintenance)

Venetoclax toxicity event	Current (cohort) dosing regimen	Modification of dosing regimen
1st event	28 days	28 days
2nd event	28 days	21 days
3rd event	21 days	14 days
4th event	14 days	discontinue

There will be no dose reductions of Venetoclax for hematologic toxicity during cycles 1 and 2. Patients who are in CRi after either cycle may temporarily discontinue Venetoclax for up to four weeks to allow for platelet or ANC recovery. Patients who enter the maintenance phase must have platelets ≥ 50 x 10^9/L and an ANC ≥ 1.0 × 10^9/L

If a dose reduction is required, please follow the guidelines in Table 7. Missed Venetoclax doses are not to be made up.

Table 8. Venetoclax Dosing Regimen Modifications

Toxicity	Occurrence	Action
Tumor Lysis Syndrome (TLS)		
TLS defined by the Cairo-Bishop criteria; see Appendix IV	Any	Implement standard TLS treatment (increase IVF). Withhold Venetoclax if TLS labs do not improve by 25% within 24 hours. Resume once TLS labs improve.
Non-Hematologic Toxicities (Consolidation and Maintenance only)		
Grade 3 or 4 non-hematologic toxicities	1st occurrence	Withhold Venetoclax. Once the toxicity has resolved to Grade 1 or baseline, resume at same dose.
	2nd and subsequent occurrences	Withhold Venetoclax. Once the toxicity has resolved to Grade 1 or baseline, resume at a reduced dosing duration (see Table 7).
Hematologic Toxicities (Maintenance only)		
Grade 4 neutropenia with or without fever; or Grade 4 thrombocytopenia	1st occurrence	Interrupt Venetoclax. To reduce infection risks associated with neutropenia, G-CSF may be administered if clinically indicated, provided there is bone marrow confirmation of leukemia clearance. Once the toxicity has resolved to Grade 2 or baseline, resume at the same dose duration (Table 7).
	2nd occurrence	Interrupt Venetoclax. Consider using G-CSF as clinically indicated. Once the toxicity has resolved to Grade 2 or baseline, follow dose duration reduction guidelines (Table 7).

	3rd and subsequent occurrences	Interrupt Venetoclax. Consider using G-CSF as clinically indicated. If toxicity remains at grade 3, may discuss with PI regarding treatment options. If grade 4 toxicity persists, remove from study (Table 7).
Consider discontinuing Venetoclax if toxicities recur despite two dose duration reductions		

6.3 Protocol Therapy Discontinuation

In addition to discontinuation due to therapy-related toxicities as outlined in section **6.1**, a subject will also be discontinued from protocol therapy and then 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) and should also be discussed with the study sponsor-investigator.

- Documented disease progression
- Unacceptable toxicity
- The treating physician believes a change of therapy would be in the best interest of the subject
- Follow-up bone marrow biopsy demonstrates either significant residual leukemic blasts or significant cytoreduction with a high percentage residual leukemic blasts, but BM study is deemed by the hematopathologist to demonstrate less than a partial response.
- Bone marrow biopsy during maintenance phase shows stable disease but the treating hematologist believes that the Venetoclax is no longer of benefit.
- DLT as defined above or if there is grade 3 neutropenia or thrombocytopenia after holding Venetoclax for four weeks at the end of cycles 1 or 2
- Patient is unable to comply with protocol requirements
- 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
- Development of a second malignancy (except for basal cell carcinoma or squamous cell carcinoma of the skin) that requires treatment which would interfere with this study.
- If protocol therapy is interrupted for ≥ 30 days.
- Lost to follow up. If a research subject cannot be located to document survival, the subject may be considered "lost to follow-up." All attempts to contact the subject must be documented.

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.

6.4 Study Stopping Rules

This is a phase I trial with a 3+3 study design. Subjects will be monitored for DLTs lasting no later than Day 56; however, if all patients in a dose cohort have recovered from DLTs prior to Day 56, dose escalation may proceed. If a DLT occurs in 1 of the 3 subjects in a given dose cohort, the dose cohort will be expanded to 6 subjects. If 2 of the total 6 subjects in any given dose cohort

experience a 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. If this occurs in cohort 1, the dose will be decreased to cohort -1 and if 2 or more DLTs are reached in dose level -1, the study will be put on hold and a protocol amendment will be considered.

7. STUDY CALENDAR & EVALUATIONS

	Screen	**Induction**[2, 3]				**Consolidation**[2,3]			**Maintenance Phase**[2,3]		**Follow up**[4]	
		Cycle 1				Cycle 2			Cycles 3+		Safety F/U	Long-term Follow up
Cycle = 28 days	-28 days[1]	Ramp up	D1	D15	D28	D1	D15	D28	D1	D15	30 days post Tx	Q 2 months (±14 days)[4]
REQUIRED ASSESSMENTS												
Informed Consent	X											
Medical history, Prior therapy, trial awareness[5]	X											
Physical exam, ECOG Performance status	X		X	X		X	X	X	X	C3	X	X
Vital signs (temp, HR, BP)	X		X	X		X	X	X	X	C3	X	X
Height (screening only), weight	X		X	X		X	X		X	C3		X
Con meds, AEs, venetoclax drug diary review[6]	X		X	X		X	X	X	X	C3	X	X
QLQ-C30, FACT-Fatigue, walk test, chair stands		X[12]						X	Q2[13]			X[12]
EKG, Echocardiogram	X											
LABORATORY ASSESSMENTS												
Pregnancy test (serum or urine) WOCBP[7]	-7d											
Hepatitis B/C and HIV screen	X											
Complete Blood Cell Count with diff (CBC)	X	X[8]	X[8]	X[8]	X	X[8]	X[8]	X	X	X	X	X
Basic Metabolic Panel (BMP), Uric Acid	X	X[8]	X[8]	X[8]		X[8]	X[8]		X	X		X
Albumin, ALT, AST, bili, alk phos, T protein, LDH	X		X[8]	X[8]		X[8]	X[8]		X	X		X
PT/INR PTT, fibrinogen	X	X[8]	X[8]	X[8]								
DISEASE ASSESSMENT												
Bone Marrow Aspirate and Biopsy[9]	-14d[1,9]				X			X	C3 (if PR) C7[9], C13[9], relapse			
TREATMENT EXPOSURE												
Gemtuzumab Ozogamicin (IV)			1,4,7			1,4						
Venetoclax (PO)		-2,-1,0	D1-28			D1-28			D1-28			
CORRELATIVE STUDIES (SPECIMEN COLLECTION)												
Buccal Swab		X[12]										
Whole Blood[10]		X[10,11]	D3[10]		X[10]			X[10]	C7[10], C13[10], relapse			
Bone Marrow Aspirate[11]	X[11,12]				X[11]			X[11]	C7[11], C13[11], relapse			
BANKING SAMPLES (SPECIMEN COLLECTION)												
Whole Blood, Serum and Plasma[14]		X			X			X	C7, C13, relapse			
FOLLOW-UP												
Survival status, subsequent therapy												X[15]

1: Screening assessment needs to be completed within 28 days. **Exception:** Bone marrow aspirate needs to be collected within 14 days prior to initiation of treatment.

2: See section 5 for treatment plan.

3: A window of ±3 days will be applied to all cycle 1 and 2 treatment visits; a ±7-day window will apply for maintenance visits.

4: Subjects without documented disease progression will be followed for disease progression every 2 months (±14 days) for 6 months. Once disease progression is documented, subjects will enter a survival follow up period: every 2 months for 6 months from the time of documented progression.

5: Medical history will capture genetic sequencing results/cytogenetics report, prior substance abuse, organ transplant, history of liver disease and family history of cancer.

6: Drug diary review will be performed on Day 1, prior to dispensing the next cycle of venetoclax.

7: For women of childbearing potential (WOCBP): urine or serum βhCG, within 7 days prior to study registration. If a urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

8: CBC should be performed daily while subjects are in the hospital. Basic metabolic panel (calcium, carbon dioxide, chloride, creatinine, glucose, potassium, sodium, urea nitrogen), coags and uric acid measurements will be performed at least every 8 hours during the ramp up and every 8-12 hours during Cycle 1 Days 1-3 to monitor for tumor lysis syndrome. Subsequently, recommend basic metabolic panel and liver function tests be performed at least three times weekly during the induction phase. Uric acid should be evaluated at further intervals at the discretion of the investigator. Recommend measuring coags weekly during the remainder of the induction phase. During consolidation, recommend measuring these labs daily while patient is in hospital and at least weekly when outpatient, at the discretion of the treating physician. During maintenance, recommend measuring these labs at least once every two weeks when outpatient, at the discretion of the treating physician.

9: Bone Marrow Aspirate and Biopsy and/or evaluation of extramedullary site with immunophenotyping, cytochemistry, and cytogenetics (karyotype ± FISH): to be completed at pre-treatment (within 14 days), Day 28 ± 3 days during cycles 1 and 2, and ± 14 days pre-treatment cycle 7 day 1 (i.e. ~6 mo), pre-treatment cycle 13 day 1 (i.e. ~12 mo), and relapse. Patients in PR at the end of consolidation should have a repeat BM assessment after one month of maintenance.

10: Whole blood to be collected pre-treatment, Cycle 1 Day +3 (±1 day), end of induction, end of consolidation, pre-treatment cycle 7 day 1 (i.e. ~6 mo), pre-treatment cycle 13 day 1 (i.e. ~12 mo), and relapse. See section 8. See Correlative Laboratory Manual (CLM) for collection, labeling, processing, and shipping instructions.

11: Bone marrow aspirate to be collected pre-treatment, end of induction, end of consolidation, pre-treatment cycle 7 day 1 (i.e. ~6 mo), pre-treatment cycle 13 day 1 (i.e. ~12 mo), and relapse. See CLM for collection, labeling, processing, and shipping instructions. Left-over samples will be banked for potential future studies, with patient consent. See section 8.

12: Pre-treatment evaluation includes buccal swab, whole blood collection, QOL (QLQ-C30, FACT-Fatigue), walk test, and chair stands. Pre-treatment bone marrow aspirate will be collected at screening. Aspirate collection for correlative studies is required prior to therapy.

13: QOL (QLQ-C30, FACT-Fatigue), walk test and chair stands to be performed pre-treatment, at the end of consolidation, every other cycle starting with Cycle 4 and throughout long term follow up.

14: Whole Blood, Serum and Plasma for banking to be collected pre-treatment, end of induction, end of consolidation, pre-treatment cycle 7 day 1 (i.e. ~6 mo), pre-treatment cycle 13 day 1 (i.e. ~12 mo), and relapse. See CLM for collection, labeling, processing, and shipping instructions.

15: It is suggested that patients should not receive HSCT conditioning within 2-3 months of last Gemtuzumab dose and that total body irradiation (TBI)/ total marrow irradiation (TMI) as part of a HSCT conditioning regimen be avoided, if possible, per discretion of the site PI.

7.1 Safety Follow-up Evaluations

A safety follow-up visit will occur when subjects permanently stop study treatment for whatever reason (toxicity, progression, or at discretion of site investigator) and will 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.

7.2 Long Term Follow-up Evaluations

All subjects will be followed until documented disease progression. Subjects who discontinue treatment for any reason without documented disease progression will be followed for disease progression every 2 months (±14 days) for 6 months.

Once disease progression is documented, subjects will enter a survival follow up period every 2 months (±14 days) for 6 months from the time of documented progression.

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

8. BIOSPECIMEN STUDIES AND PROCEDURES

8.1 Correlative Studies

8.1.1 Longitudinal assessment of genomic minimal residual disease (MRD) with droplet digital PCR (ddPCR) will determine the kinetics and depth of response and its association with RFS and OS. ddPCR is a novel technology capable of detecting gene mutations in as few as 1 in 50,000 cells, providing a highly sensitive platform for AML MRD measurement.[42] DNA extracted from pre-treatment patient bone marrow or peripheral blood mononuclear cells (PBMC) will undergo targeted deep sequencing of recurrently mutated genes in AML and/or whole exome sequencing to identify somatically-acquired leukemia-associated gene mutations. These mutations will be confirmed to be somatic by Sanger resequencing of both tumor and germline (derived from buccal swab) DNA. ddPCR will then detect these mutations in serial post-treatment samples (4 time points: end of induction, end of consolidation, and at 6 months and 12 months) using genomic DNA from bone marrow and/or PBMC as well as plasma-based cell-free DNA to determine the presence of genomic MRD at each time point. Both the rate and maximal level of reduction of leukemia-associated gene mutations will be correlated with RFS and OS. The measurement of multiple genes per patient will allow assessment of clonal dynamics in the MRD state and the relative clearance of leukemic versus pre-leukemic cells (defined as clones containing all gene mutations identified pre-treatment or those containing only a subset of those gene mutations, respectively). These experiments will be performed at the laboratory of Dr. Brian Parkin.

8.1.2 Association of CD33 cell-surface expression and *CD33* single nucleotide polymorphisms (SNP) and somatic mutations with response to the combination of GO and Venetoclax will be determined based on serial genomic MRD levels, CR, and RFS. The presence of splice acceptor SNPs that lead to alterative splicing of *CD33* that eliminates the IgV portion of the molecule to

which GO binds has recently been shown to adversely influence clinical effectiveness of GO in trials including GO alone or in combination with chemotherapy.[11] In order to study the impact of such *CD33* variants in this trial, all coding exons and adjacent intronic sequences of *CD33* will be sequenced from genomic DNA extracted from pre-treatment samples and paired buccal swabs to identify germline SNPs and acquired somatic mutations. Similar sequencing will be performed on any relapsed specimens as well to determine emergence of secondarily-acquired *CD33* mutations. The presence of isoform altering mutations will be confirmed with isoform specific qPCR of mRNA extracted from pre-treatment samples. Finally, cell surface expression of CD33 isoforms including or lacking the IgV region will be determined using flow cytometry with isoform specific antibodies (anti-CD33 clones P67.6 and HIM3-4, respectively).[43] These experiments will be performed at the laboratory of Dr. Brian Parkin.

8.1.3 Identification of clonal evolution and secondarily acquired mutations in refractory and relapsed patient samples may reveal mechanisms underlying treatment failure. For patients who do not respond to the induction and consolidation cycles of the investigational therapy or who relapse after initial response, whole exome sequencing may be performed on germline controls (derived from buccal swabs), pre-treatment tumor samples, and tumor samples obtained at the time of treatment failure in order to identify the presence of primary or secondary acquisition of mutations that may be related to the mechanisms of treatment failure. For relapsed patients, ddPCR will be used to track mutations acquired or enriched in the relapse clone into the pre-treatment and CR samples in order to determine if the relapsed clone acquired the mutations after treatment or if a minor preexisting clone was enriched as a result of treatment. These experiments will be performed at the laboratory of Dr. Brian Parkin.

8.1.4 BCL-2 Family Protein Analysis prior to, and after Venetoclax exposure may reveal adaptive changes in anti-apoptotic protein expression. Leukocytes isolated from pretreatment and Day+3 (C1D3) peripheral blood will be analyzed by semi-quantitative Western blot analysis for BCL-2, BCL-XL and MCL-1 expression.[16] If sufficient samples are available then PBMNC samples from pretreatment and Day+3 will also be permeabilized, incubated with fluorescent antibodies to BCL-2, BCL-XL and MCL-1, and expression measured by flow cytometry.[20] Analysis will also be performed on relapse samples (if this occurs). These experiments will be performed at the University of Illinois in the laboratory of Dr. John Quigley.

8.1.5. BH3 profiling predicts response to therapy.
Thawed BM samples from pretreatment and, potentially, relapse samples will be permeabilized, incubated with various BH3 peptides and subsequently mitochondrial cytochrome C release measured by flow cytometry analyses.[16] These experiments will be performed at the University of Illinois in the laboratory of Dr. John Quigley.

8.1.6 Quality of life, fatigue, and physical function will be assessed at baseline and every other month for one year to measure the effects of therapy.[32] Quality of life (QOL) will be assessed using the European Organization for the Research and Treatment of Cancer 30 item questionnaire (EORTC QLQ-C30). The questionnaire is scored from 0 to 100, higher scores positively correlate with a better QOL. The Functional Assessment of Cancer Therapy Fatigue (FACT-Fatigue) questionnaire consists of 13 items with scores ranging from 0 to 52, higher scores positively

correlate with less fatigue. Objective physical measures will consist of a two-minute walk test, and timed chair stands. See Appendix V for sample questionnaires.

8.2 Source and Timing of Biospecimen Collections

8.2.1 Buccal swab:

Patients will have buccal mucosal cells collected prior to treatment using two buccal swabs to provide germline DNA. See Correlative Laboratory Manual (CLM) for collection, labeling, processing, and shipping instructions.

8.2.2 Bone marrow aspirate:

Bone marrow aspirate material will be collected will be collected prior to treatment, at the end of the induction cycle (Cycle 1 Day 28 ± 3 days), at the end of the consolidation cycle (Cycle 2 Day 28 ± 3 days), at pre-treatment cycle 7 day 1 (i.e. ~6 months after initial treatment) (± 14 days), at pre-treatment cycle 13 day 1 (i.e. ~12 months after initial treatment) (± 14 days), and at any time of suspected relapse. Leukocytes from the bone marrow aspirate will be isolated by Ficoll gradient centrifugation and cryopreserved for use in the correlative studies described. See CLM for collection, labeling, processing, and shipping instructions.

8.2.3 Peripheral blood:

Peripheral blood samples will be collected prior to treatment, at Cycle 1 Day +3 (± 1 day), at the end of the induction cycle (Cycle 1 Day 28 ± 3 days), at the end of the consolidation cycle (Cycle 2 Day 28 ± 3 days), at pre-treatment cycle 7 day 1 (i.e. ~6 months after initial treatment) (± 14 days), at pre-treatment cycle 13 day 1 (i.e. ~12 months after initial treatment) (± 14 days), and at any time of suspected relapse. Correlative peripheral blood samples will preferentially be obtained during clinically indicated peripheral blood draws. Plasma will be isolated and frozen, and leukocytes will be isolated by Ficoll gradient centrifugation and cryopreserved for use in the correlative studies described. See CLM for collection, labeling, processing, and shipping instructions.

8.3 Storage of Biospecimens

8.3.1 Specimen Processing and Storage:

Buccal swabs will be stored at -20° until DNA extraction. Plasma will be isolated from peripheral blood tubes and stored in aliquots at -80°. Bone marrow aspirates and peripheral blood samples will undergo Ficoll gradient centrifugation and mononuclear cells with be aliquoted and cryopreserved in liquid nitrogen. These samples will be stored in the central lab for use in the correlative studies outlined in this protocol as well as for future research.

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

8.4 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.

8.5 Banking Samples for Future Unspecified Research

Subject consent will be obtained to collect additional blood 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.

8.6 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.

9. CRITERIA FOR DISEASE EVALUATION

9.1 Definitions of Response to Treatment in AML

Disease will be assessed according to the International Working Group Criteria[31].

Category	Definition
Treatment Success categories	
Complete remission (CR)	Bone marrow blasts < 5%; absence of blasts with Auer rods; absence of extramedullary disease; absolute neutrophil count > 1.0 x 10^9/L (1000/µL); platelet count > 100 x 10^9/L (100,000/µL); independent of red cell transfusions
CR with incomplete recovery (CRi)	All CR criteria except for residual neutropenia < 1.0 x 10^9/L (1000/µL) or thrombocytopenia < 100 x 10^9/L (100,000/µL)
Partial remission (PR)	All hematologic criteria of CR; decrease of bone marrow blast percentage to 5% to 25%; and decrease of pretreatment bone marrow blast percentage by at least 50%
Treatment Failure categories	
Resistant Disease	Failure to achieve CR or CRi or failure to achieve CR, CRi, or PR; only includes patients surviving ≥ 7 days following completion of initial treatment, with evidence of persistent leukemia by blood and/or bone marrow examination
Death in aplasia	Deaths occurring ≥ 7 days following completion of initial treatment while cytopenic; with an aplastic or hypoplastic bone marrow obtained within 7 days of death, without evidence of persistent leukemia
Death from indeterminate cause	Deaths occurring before completion of therapy, or < 7 days following its completion; or deaths occurring ≥ 7 days following completion of initial therapy with no blasts in the blood, but no bone marrow examination available

Relapse	Bone marrow blasts ≥ 5%; or reappearance of blasts in the blood; or development of extramedullary disease

Adapted from International Working Group Criteria[31]

In patients with abnormal cytogenetics at diagnosis, CR (or CRi) will be delineated as above plus evidence of complete cytogenetic response (also known as CRc)—defined as a reversion to normal cytogenetics.

9.2 Definitions of Survival Endpoints for AML

Overall Survival	All evaluable patients entered into trial; measured from the date of entry to the date of death from any cause; patients not known to have died at last follow-up are censored on the date they were last known to be alive
Relapse-free Survival	Only patients achieving CR or CRi; measured from the date of achievement of a remission until the date of relapse or death from any cause; patients not known to have relapsed or died at last follow-up are censored on the date they were last examined
Event-free Survival	All patients entered into trial; measured from the date of entry to the date of treatment failure, disease relapse, or death from any cause; patients not known to have any of these events are censored on the date they were last examined

Adapted from European LeukemiaNet.[44]

10. DRUG INFORMATION

10.1 Venetoclax (ABT-199)

10.1.1 Pharmacological Properties and FDA-Approved Indication

Venetoclax is a BCL-2 inhibitor indicated for the treatment of patients with chronic lymphocytic leukemia (CLL) with 17p deletion, as detected by an FDA approved test, who have received at least one prior therapy.

10.1.2 How Supplied

AbbVie will supply Venetoclax at no charge to subjects participating in this clinical trial.

Drug Packaging configurations: 10mg 16 tablets/card, 50mg 1×8 blister card, 100mg 1×8 blister cards, 100mg 2×8 blister cards, 100mg 120 count bottles.

Venetoclax 10 mg film-coated tablets are round, biconvex shaped, and pale yellow.

Venetoclax 50 mg film-coated tablets are oblong, biconvex shaped, and beige.

Venetoclax 100 mg film-coated tablets are oblong, biconvex shaped, and pale yellow.

10.1.3 Preparation

Venetoclax is taken 1 time a day with a meal and water at about the same time each day.

10.1.4 Storage and Stability

Store at 15 to 25 degrees C (59-77 degrees F)

10.1.5 Handling and Disposal

Subjects will be instructed to do the following when given Venetoclax to take home:

- Keep Venetoclax in a safe place, away from other family members' medications and away from any food or drinks.
- Store the medication at room temperature in a dry location – avoid storing medication in the bathroom.
- Store Venetoclax in its original container, which may be a blister pack
- Keep the medication out of reach from children and pets.
- Return damaged or unused Venetoclax to the study physician

10.1.6 Dispensing

Venetoclax must be dispensed only from official study sites and to eligible subjects under the supervision of the site investigator. Venetoclax 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.

10.1.7 Adverse events

The most common adverse reactions (≥20%) in clinical trials were neutropenia, diarrhea, nausea, anemia, upper respiratory tract infection, thrombocytopenia, and fatigue. Warnings include Tumor Lysis Syndrome and neutropenia. Refer to the package insert for a comprehensive list of adverse events.

Please refer to the latest version of the prescribing information which can be found at *http://www.accessdata.fda.gov/scripts/cder/drugsatfda/*, and/or on the manufacturer's website.

10.2 Gemtuzumab Ozogamicin (MYLOTARG™)

10.2.1 Pharmacological Properties and FDA-Approved Indication

Gemtuzumab Ozogamicin is a CD33-directed antibody-drug conjugate indicated for:

- treatment of newly-diagnosed CD33-positive acute myeloid leukemia (AML) in adults
- treatment of relapsed or refractory CD33-positive AML in adults and in pediatric patients 2 years and older

10.2.2 How Supplied

Pfizer will supply Gemtuzumab Ozogamicin at no charge to subjects participating in this clinical trial.

MYLOTARG (Gemtuzumab Ozogamicin) for Injection is a white to off-white lyophilized cake or powder supplied in a carton (NDC 0008-4510-01) containing one 4.5 mg single-dose vial.

10.2.3 Preparation

Use appropriate aseptic technique for the reconstitution and dilution procedures. Protect the reconstituted and diluted MYLOTARG solution from light.

Reconstitution

- MYLOTARG is a cytotoxic drug. Follow applicable special handling and disposal procedures.[1]
- Calculate the dose (mg) and number of vials of MYLOTARG required.
- Prior to reconstitution, allow drug product vials to reach ambient temperature for approximately 5 minutes.
- Reconstitute each vial with 5 mL of Sterile Water for Injection, USP to obtain a concentration of 1 mg/mL of MYLOTARG that delivers 4.5 mL (4.5 mg).
- Gently swirl the vial to aid dissolution. DO NOT SHAKE.
- Inspect the reconstituted solution for particulates and discoloration. The reconstituted solution may contain small white to off-white, opaque to translucent, and amorphous to fiber-like particles.
- MYLOTARG contains no bacteriostatic preservatives.
- Use reconstituted solution immediately or after being refrigerated at 2–8°C (36–46°F) for up to 1 hour. Protect from light. Do not freeze.

Dilution

- Calculate the required volume of the reconstituted solution needed to obtain the appropriate dose according to patient body surface area. Withdraw this amount from the vial(s) using a syringe. Protect from light. Discard any unused reconstituted solution left in the vial.

Doses must be mixed to a concentration between 0.075 mg/mL to 0.234 mg/mL according to the following instructions:

- Doses less than 3.9 mg must be prepared for administration by syringe. Add the reconstituted MYLOTARG solution to a syringe with 0.9% Sodium Chloride Injection to a final concentration between 0.075 mg/mL to 0.234 mg/mL. PROTECT FROM LIGHT.
- Doses greater than or equal to 3.9 mg are to be diluted in a syringe or an IV bag in an appropriate volume of 0.9% Sodium Chloride Injection to ensure a final concentration between 0.075 mg/mL to 0.234 mg/mL. PROTECT FROM LIGHT.
- Gently invert the infusion container to mix the diluted solution. Do not shake.
- Following dilution with 0.9% Sodium Chloride Injection, MYLOTARG solution should be infused immediately. If not used immediately, store at room temperature (15–25°C; 59–77°F) for up to 6 hours, which includes the 2-hour infusion time and 1-hour, if needed, to allow the refrigerated diluted solution to equilibrate to room temperature. The diluted solution can be refrigerated at 2–8°C (36–46°F) for up to 12 hours which includes up to 1-hour in the vial post-reconstitution. Protect from light and do not freeze.

Administration

- Use an in-line 0.2 micron polyethersulfone (PES) filter for infusion of MYLOTARG.
- Protect the intravenous bag from light using a light-blocking cover during infusion. The infusion line does not need to be protected from light.
- Infuse the diluted solution over 2 hours.
- Do not mix MYLOTARG with, or administer as an infusion with, other medicinal products.

10.2.4 Storage and Stability

Refrigerate (2–8°C; 36–46°F) MYLOTARG vials and store in the original carton to protect from light. Do not freeze.

10.2.5 Handling and Disposal

MYLOTARG is a cytotoxic drug. Follow applicable special handling and disposal procedure.

10.2.6 Dispensing

MYLOTARG must be dispensed only from official study sites and to eligible subjects under the supervision of the site investigator. MYLOTARG 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 study subjects.

10.2.7 Adverse events

The most common adverse reactions (greater than 15%) in clinical trials were hemorrhage, infection, fever, nausea, vomiting, constipation, headache, increased AST, increased ALT, rash, and mucositis. Warnings include infusion-related reactions and hemorrhage. Refer to the package insert for a comprehensive list of adverse events.

Please refer to the latest version of the prescribing information that can be found at *http://www.accessdata.fda.gov/scripts/cder/drugsatfda/*, and/or on the manufacturer's website.

11. ADVERSE EVENTS

11.1 Definitions

11.1.1 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.

11.1.2 Serious Adverse Event (SAE)

An SAE is an adverse event that:

- Results in death.

- 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.

11.1.3 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.

11.1.4 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)

11.2 Reporting

11.2.1 Adverse Events

- Adverse events (AEs) will be recorded from the time of consent until 30 days after treatment discontinuation of study drugs or until a new anti-cancer treatment starts, whichever occurs first.

- AEs will be recorded regardless of whether or not the event(s) are considered related to the study drugs.
- 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 drugs will be followed until resolution to ≤ Grade 1 or baseline, deemed clinically insignificant, and/or until a new anti-cancer treatment starts, whichever is earlier.

11.2.2 Reporting of Pregnancy

Although pregnancy is not considered an adverse event, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them), including the pregnancy of a male subject's female partner that occurs during the trial or within 120 days of completing the trial, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier. All subjects and female partners of male subjects who become pregnant must be followed to the completion/termination of the pregnancy. All pregnancies must be reported within 24 hours to BTCRC AHQ on the BTCRC Pregnancy Reporting form (See Documents/Info tab in the EDC).

Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage, and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

Such events must be reported within 24 hours to BTCRC AHQ on the BTCRC Pregnancy Reporting form (See Documents/Info tab in the EDC). BTCRC AHQ will report the event within 24 hours to Pfizer Global Safety (Attn: Worldwide Product Safety; FAX 1-866-997-8322) and AbbVie (Fax 1-866-997-8322; PPDINDPharmacovigilance@abbvie.com).

11.2.3 Site Requirements for Reporting SAEs and Non-Serious Events of Clinical Interest to BTCRC Administrative Headquarters

- SAEs will be reported from time of signed informed consent until 30 days after discontinuation of study drug(s) or until a new anti-cancer treatment starts, whichever occurs first.
- SAEs will be reported on the SAE Submission Form and entered in the SAE tab in the EDC system **within 24 hours** of discovery of the event.
- SAEs will be reported whether or not they are related 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.
- Non-serious Events of Clinical Interest (including tumor lysis syndrome) will be reported to BTCRC AHQ and will be handled in the same manner as SAEs.
- Additionally, any serious adverse event, considered by an investigator to be related to either study drug, which is brought to the attention of the investigator at any time outside of the 30-day time period specified in the previous paragraph, also must be reported immediately to BTCRC AHQ.

The site will electronically submit the completed BTCRC SAE Submission Form (see Documents/Info tab in the EDC) to BTCRC AHQ within 24 hours of discovery of the event (safety@hoosiercancer.org). The investigator is responsible for informing the IRB and/or the Regulatory Authority of the SAE as per local requirements.

The original copy of the BTCRC 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 submit a follow up SAE Submission Form within a reasonable timeframe to BTCRC AHQ electronically to safety@hoosiercancer.org.

11.2.4 BTCRC AHQ Requirements for Reporting SAEs to Pfizer

BTCRC AHQ will submit all SAEs received from sites to Pfizer within 24 hours of receipt of the BTCRC SAE Submission Form and to regulatory authorities (FDA) per federal guidelines.

BTCRC AHQ will submit all SAE reports and any other relevant safety information to:

Pfizer U.S. Clinical Trial Department
Facsimile number: 1-866-997-8322

BTCRC AHQ will provide follow-up information to Pfizer as it is received from a site.

11.2.5 BTCRC AHQ Requirements for Reporting SAEs to AbbVie

For any subject receiving Study Product, BTCRC AHQ will submit all SAEs received from sites to AbbVie within 24 hours of receipt of the BTCRC SAE Submission Form and to regulatory authorities (FDA) per federal guidelines.

BTCRC AHQ will submit all SAE reports and any other relevant safety information to AbbVie at:
Facsimile number: +1 (847)-938-0660
Email: PPDINDPharmacovigilance@abbvie.com

BTCRC AHQ will provide follow-up information to AbbVie as it is received from a site.

11.2.6 BTCRC AHQ Responsibilities for Reporting SAEs to FDA

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

BTCRC 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, BTCRC AHQ will submit a copy of these reports to Pfizer and AbbVie at the time of submission to FDA.

11.3 Sponsor-Investigator Responsibilities

BTCRC 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.

11.4 IND Safety Reports Unrelated to This Trial

AbbVie and Pfizer 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 BTCRC AHQ, site investigators (or designees) are responsible for submitting these safety reports to their respective IRBs, as per their IRB policies.

12. STATISTICAL METHODS

12.1 Study Design

This will be a single arm, open-label multicenter study of the combination of Venetoclax and Gemtuzumab Ozogamicin (GO) in patients with relapsed or refractory AML. It will be an initial dose-escalation phase Ib study (Cohorts 1-3), using a 3+3 design to determine whether there is a dose-limiting toxicity. There will be a 28-day induction and then a 28-day consolidation cycle, followed by a maintenance phase.

12.2 Endpoints

12.2.1 Definition of Primary Endpoint

- Dose-limiting toxicity, defined as an adverse event related (possible, probably, or definite) to Venetoclax and/or Gemtuzumab fulfilling one of the following criteria:
  - Hematologic toxicity: treatment-related grade 3 or worse neutropenia and/or thrombocytopenia due to bone marrow hypocellularity present at the end of cycle one (day 28) with an additional 28 days allowed for count recovery (i.e. present at day 56); specifically grade 3 or worse neutropenia or thrombocytopenia with the bone marrow documented to be free of leukemic infiltration. Note: patients who enter the study with grade 3 or worse cytopenias will not be evaluable for hematologic dose-limiting toxicities.
  - Non-hematologic toxicity: any grade 3 or worse treatment-related toxicity occurring within the first cycle (excluding grade 3-4 infections during cycle one).

12.2.2 Definition of Secondary Endpoints

- Overall response rate (CR/CRi), as defined by the revised IWG criteria[24]. See Section 9.1.
- Anti-leukemic activity (CR/CRi/PR), as defined by the revised IWG criteria[24]. See Section 9.1.
- Adverse effects will be characterized using the CTCAE v5 criteria.
- Relapse-free survival (RFS), event-free survival (EFS, and overall survival (OS). See Section 9.2

- The European Organization for the Research and Treatment of Cancer 30 item questionnaire (EORTC QLQ-C30), The Functional Assessment of Cancer Therapy Fatigue (FACT-Fatigue) questionnaire, two-minute walk test, and timed chair stands will be performed at trial entry and every other month for 1 year.[32]

12.2.3 Definition of Exploratory Endpoints

- Genomic MRD with droplet digital PCR (ddPCR) will be measured to determine kinetics of response and the association with relapse-free survival at the end of cycles 1 and 2, at 6 months and 12 months, and if there is evidence of relapse. (Sub-PI is Dr. Brian Parkin [see ref. 33].)
- CD33 DNA polymorphisms will be measured at presentation and at relapse.
- Leukemic blast CD33 expression will be measured at presentation and at relapse.
- "BH3 profiling"[34] of AML blasts will be performed at presentation and at relapse.
- BCL-2, BCL-XL, and MCL-1 protein levels in PB or BM blasts will be measured at presentation and after 6 days (i.e., Cycle 1 Day +3) of Venetoclax therapy.

12.3 Sample Size and Accrual

A standard "3 + 3" design will be used to establish the maximum tolerated dose. The maximum tolerated dose is defined as the dose level at which fewer than 33% of subjects experience a dose limiting toxicity. An expansion cohort of 6 patients will be carried out at the maximum tolerated dose, which will be the recommended Phase II dose.

This study is a traditional '3 + 3' design without de-escalation, a specified A + B dose finding study with parameters of A = B = 3, $c_L = 0$, $c_U = 2$, and $C_U = 1$. The associated target quantiles for our study are $\Gamma_A = 0.35$ and $\Gamma_A = 0.26$. Hence, the dose selected by this design has a toxicity rate below 0.26 and above $C_U/(A + B)$ on average. In simulation studies, the apparent selected dose with our targeted N=18-24 (n=9-18, plus 6) selects a dose with toxicity rate near 0.2. In this scenario, the '3 + 3' design $\Gamma_U = (C_U + 1)/(A + B) = 2/(3 + 3) = 0.33$ is our upper bound. To evaluate the quality of estimation of the target dose per average sample size increase, we reviewed simulation studies of this design under conditions of A = B = 3 with values of change (Δ) of either 0.35 or 0.70. Within the average sample size range of 16.2 to 21.8, between 78% and 89% correct dose recommendations were achieved, where tendencies of higher Δ resulted in greater percent correct dose recommendations [see ref. 45 for further review].

12.4 Assessment of Safety

Patients evaluable for toxicity are those who receive at least one dose of Venetoclax and one dose of GO. The NCI Common Terminology Criteria for Adverse Events (CTCAE) v5 will be used to grade adverse events per the defined Study Calendar for toxicity assessment schedule.

12.5 Assessment of Efficacy

All subjects with disease who received at least one dose of study drug and have had their disease re-evaluated (or die beforehand), will be included in the efficacy analysis. Per the analysis plan described in section 12.8.2 for all time-to-event outcomes (e.g., survival) identified as secondary endpoints, cumulative incidence and hazard functions will be estimated as conditional survival probabilities until first meeting the defined outcome criteria for efficacy:

- ORR (CR/CRi) and anti-leukemic activity (CR/CRi/PR) after 2 cycles. See Section 9.1.
- Relapse-free and event-free survival
- Overall survival in subjects

12.6 Statistical Analysis Plans

12.6.1 Analysis Plans for Primary Objective

DLTs will be presented by dose of Venetoclax when administered with Gemtuzumab Ozogamicin.

12.6.2 Analysis Plans for Secondary Objectives

Descriptive characteristics of all adverse effects of GO in combination with Venetoclax (using CTCAE v5 criteria) will be reported; and the efficacy of the combination in achieving ORR (includes CR/CRi) and anti-leukemic activity (includes CR/CRi/PR), as defined by the revised IWG criteria[24]), RFS, EFS, and OS in subjects. The effects of therapy on quality of life, fatigue, and physical function at D0 (trial entry) and the end of every other month for 1 year (see section 8.1) will be reported and analyzed using generalized linear modeling.

All time-to-event outcomes (e.g. survival) will be calculated using Kaplan Meier methods and exploratory log-rank tests will be used to compare treatment effect between groups based on genomic MRD, CD33 DNA polymorphisms, BH3 profiling and anti-apoptosis protein levels in PB MBCs at presentation and after 1 week of Venetoclax.

Sample size permitting: Hazard ratios (HR) and 95% CIs will be produced. Time-to-event outcomes with a competing or semi-competing risk (e.g. CIR) will be analyzed using cumulative incidence curves and exploratory comparisons between arms will be made using Grey's test. Rate ratios and 95% CIs will be presented. Chi squared tests will be used to assess the treatment effect between cohorts for all categorical variables and t-tests or Mann Whitney tests will be applied for continuous measures as appropriate.

12.6.3 Other Planned Analyses

Descriptive characteristics of patients will be reported in tabular form along with the dates of trial opening and closing, indicating the total number of patients enrolled and accounting for all patients considered for entry (flow diagram). Patients found to be ineligible will be indicated along with their reasons for ineligibility and whether they received any study therapy. Of the included subjects, the number of patients who were evaluable for toxicity and response (secondary objectives, see section **12.2.2**) will be reported.

Drug administration by dose level will be reported in tables detailing the total number of patients treated, number of doses or cycles received at dose level, and the number of incomplete cycles, including brief descriptions of what happened in dose escalation if intermediate dose levels or deviations from study protocol resulted. Any dose holds, reductions, and delays, and permanent discontinuation of study treatments will be summarized with reasons for each. Finally, summaries of dose intensity will be estimated, including how many patients were able to receive each planned dose intensity of Venetoclax administered with Gemtuzumab Ozogamicin.

Tables of adverse effects displaying the worst grade by dose level will be reported, including columns or each grade and "any grade" adverse effects. Separate tables report hematological and non-hematological effects will be reported according to dose escalation.

Descriptive tables reporting frequencies and means or medians of scores of the EORTC QLQ-C30 and FACT-Fatigue questionnaire, two-minute walk test, and timed chair stand will be created for D0 (trial entry) and subsequent months of follow up. Generalized linear modeling of continuous scores or ordinal values accounting for correlation and repeated will be performed to determine differences between groups

and characteristics considered to be potential confounding factors. Given a threshold value indicating a substantial degree of the outcome (i.e., quality of life, fatigue and physical function), generalized estimating equation models with a binomial distribution and unstructured correlation covariance will be used to produce odds ratios (OR) and robust 95% CI for the relation of dose and clinical characteristics to each outcome.

13. TRIAL MANAGEMENT

13.1 Data and Safety Monitoring Plan (DSMP)

The study will be conducted in accordance with the University of Illinois Cancer Center's Data and Safety Monitoring Plan (DSMP). The University of Illinois Cancer Center Data Safety and Monitoring Committee (DSMC) will review and make recommendations on this trial. BTCRC AHQ will provide the University of Illinois Cancer Center DSMC with periodic data reports to comply with the UICC DSMC review requirements.

In addition, BTCRC AHQ oversight activities include:

- Review of all adverse events requiring expedited reporting as defined in the protocol.
- Provide trial accrual progress, safety information, and data summary reports to the sponsor-investigator.
- Submit data summary reports to the lead institution DSMC for review as per their DSMP.

13.2 University of Illinois Cancer Center Data Safety Monitoring Committee

BTCRC AHQ will provide the UICC DSMC with the following:

- Adverse event summary report
- Audit results, if applicable
- Data related to stopping/decision rules described in study design
- Study accrual patterns
- Protocol deviations

The University of Illinois Cancer Center DSMC will review study data every quarter. Documentation of DSMC reviews will be provided to the sponsor-investigator and BTCRC AHQ. Issues of immediate concern by the DSMC will be brought to the attention of the sponsor-investigator and other regulatory bodies as appropriate. The sponsor-investigator will work with BTCRC AHQ to address the DSMC's concerns.

13.3 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 BTCRC AHQ or its designee.

The trial site may also be subject to quality assurance audit by Pfizer, AbbVie, or their designee as well as inspection by appropriate regulatory agencies.

13.4 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. The sponsor-investigator has delegated responsibility to BTCRC 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.

14. DATA HANDLING AND RECORD KEEPING

14.1 Data Management

BTCRC 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. Other study institutions will be given a password to directly enter their own data onto the web site via electronic case report forms (eCRFs). BTCRC 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.

14.2 Case Report Forms and Submission

An electronic case report form (eCRF) is required and must be completed for each included subject. The completed dataset is housed at BTCRC 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 permission from the sponsor-investigator and BTCRC AHQ. After the initial publication, the complete data set will be available to all BTCRC institutions.

14.3 Record Retention

To enable evaluations and/or audits from Health Authorities/BTCRC AHQ, the investigator agrees to keep records, including the identity of all participating 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. To comply with international regulations, the records should be retained by the investigator in compliance with regulations.

During data entry, range and missing data checks will be performed on-line. The checks to be performed will be documented in the Data Monitoring Plan for 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. This will be done on an ongoing basis.

14.4 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, BTCRC AHQ, Pfizer, AbbVie, 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.

15. ETHICS

15.1 Institutional Review Board (IRB) Approval

The final study protocol, including the final version of the informed consent form, must be approved in writing by an IRB. The investigator must submit written approval by the IRB to the BTCRC AHQ office before he or she can enroll any subject 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 annually, as local regulations require.

Progress reports and notifications of serious unexpected adverse drug reactions will be provided to the IRB according to local regulations and guidelines.

15.2 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.

15.3 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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Appendix I – ECOG Performance Status

ECOG Score	Performance Status
0	Asymptomatic
1	Symptomatic, fully ambulatory
2	Symptomatic, in bed < 50% of the day
3	Symptomatic, in bed > 50% of the day but not bedridden
4	Bedridden
5	Dead

Adapted from ECOG Definitions[46]

Appendix II – New York Heart Association Classification

Class	Functional Capacity	Objective Assessment
I	Patients with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.	No objective evidence of cardiovascular disease.
II	Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of minimal cardiovascular disease.
III	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of moderately severe cardiovascular disease.
IV	Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.	Objective evidence of severe cardiovascular disease.

Adapted from the American Heart Association Guidelines[47]

Appendix III – Prohibited and Cautionary Medications

Excluded throughout the study
Strong CYP3A inducers – avasimibe, carbamazepine, enzalutamine, mitotane, phenytoin, rifampin, St. John's wort **Moderate CYP3A inducers** - bosentan, efavirenz, etravirine, modafinil, nafcillin
Excluded during ramp-up phase and Cautionary at the Cohort Designated Dose
Strong CYP3A inhibitors - boceprevir, clarithromycin, cobicistat, conivaptan, danoprevir/ritonavir, elvitegravir/ritonavir, idelalisib, indinavir, itraconazole, ketoconazole, mibefradil, lopinavir/ritonavir, nefazodone, nelfinavir, ritonavir, paritaprevir/ritonavir combinations, posaconazole, saquinavir, telaprevir, telithromycin, tipranavir/ritonavir, voriconazole **Moderate CYP3A inhibitors -** amprenavir, aprepitant, atazanavir, cimetidine, ciprofloxacin (*not levofloxacin*), clotrimazole, crizotinib, cyclosporine, darunavir/ritonavir, diltiazem[1], erythromycin, fluconazole (*>200mg*), fosamprenavir, imatinib, isavuconazole, tofisopam, verapamil
Cautionary
Warfarin **P-gp substrates** Aliskiren, ambrisentan, colchicine, dabigatran etexilate, digoxin, everolimus, fexofenadine, lapatinib, loperamide, maraviroc, nilotinib, ranolazine, saxagliptin, sirolimus, sitagliptin, talinolol, tolvaptan, topotecan **BCRP substrates** Methotrexate, mitoxantrone, irinotecan, lapatinib, rosuvastatin, sulfasalazine, topotecan **OATP1B1/1B3 substrates** Atrasentan, atorvastatin, ezetimibe, fluvastatin, glyburide, rosuvastatin, simvastatin acid, pitavastatin, pravastatin, repaglinide, telmisartan, valsartan, olmesartan **BCRP inhibitors** Geftinib

Appendix IV – Cairo Bishop Guidelines

Cairo-Bishop definition of laboratory tumor lysis syndrome: Either the presence of two or more of the following laboratory changes within 3 days before or seven days after cytotoxic therapy ***OR*** a 25% change above or below normal.		
Element	**Value**	**Change from baseline**
Uric Acid	≥476 μmol/L (8mg/dL)	or 25% increase
Potassium	≥6.0 mmol/L (or 6mEq/L)	or 25% increase
Phosphorus	≥1.45 mmol/L (4.5 mg/dL) for adults	or 25% increase
Calcium	≤1.75 mmol/L (7mg/dL)	or 25% decrease

Adapted from the Guidelines for the management of pediatric and adult tumor lysis syndrome: an evidence-based review[48]

Appendix V – Quality of Life and Fatigue Questionnaires

EORTC QLQ-C30 (version 3)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Please fill in your initials: ⌊_⌋⌊_⌋⌊_⌋
Your birthdate (Day, Month, Year): ⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋
Today's date (Day, Month, Year): 31 ⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋

		Not at All	A Little	Quite a Bit	Very Much
1.	Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?	1	2	3	4
2.	Do you have any trouble taking a long walk?	1	2	3	4
3.	Do you have any trouble taking a short walk outside of the house?	1	2	3	4
4.	Do you need to stay in bed or a chair during the day?	1	2	3	4
5.	Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the past week:		Not at All	A Little	Quite a Bit	Very Much
6.	Were you limited in doing either your work or other daily activities?	1	2	3	4
7.	Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8.	Were you short of breath?	1	2	3	4
9.	Have you had pain?	1	2	3	4
10.	Did you need to rest?	1	2	3	4
11.	Have you had trouble sleeping?	1	2	3	4
12.	Have you felt weak?	1	2	3	4
13.	Have you lacked appetite?	1	2	3	4
14.	Have you felt nauseated?	1	2	3	4
15.	Have you vomited?	1	2	3	4

Please go on to the next page

During the past week:		Not at All	A Little	Quite a Bit	Very Much
16.	Have you been constipated?	1	2	3	4
17.	Have you had diarrhea?	1	2	3	4
18.	Were you tired?	1	2	3	4
19.	Did pain interfere with your daily activities?	1	2	3	4
20.	Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21.	Did you feel tense?	1	2	3	4
22.	Did you worry?	1	2	3	4
23.	Did you feel irritable?	1	2	3	4
24.	Did you feel depressed?	1	2	3	4
25.	Have you had difficulty remembering things?	1	2	3	4
26.	Has your physical condition or medical treatment interfered with your family life?	1	2	3	4
27.	Has your physical condition or medical treatment interfered with your social activities?	1	2	3	4
28.	Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you

29. How would you rate your overall health during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

30. How would you rate your overall quality of life during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

Functional Assessment of Chronic Illness Therapy (FACIT) Fatigue Scale (Version 4)

Below is a list of statements that other people with your illness have said are important. **Please circle or mark one number per line to indicate your response as it applies to the past 7 days.**

		Not At All	A Little Bit	Somewhat	Quite a Bit	Very Much
1	I feel fatigued	0	1	2	3	4
2	I feel weak all over	0	1	2	3	4
3	I feel listless ("washed out")	0	1	2	3	4
4	I feel tired	0	1	2	3	4
5	I have trouble starting things because I am tired	0	1	2	3	4
6	I have trouble finishing things because I am tired	0	1	2	3	4
7	I have energy	0	1	2	3	4
8	I am able to do my usual activities	0	1	2	3	4
9	I need to sleep during the day	0	1	2	3	4
10	I am too tired to eat	0	1	2	3	4
11	I need help doing my usual activities	0	1	2	3	4
12	I am frustrated by being too tired to do the things I want to do	0	1	2	3	4
13	I have to limit my social activity because I am tired	0	1	2	3	4

Scoring: Items are scored as follows: 4=Not At All; 3=A Little Bit; 2=Somewhat; 1=Quite A Bit; 0=Very Much, EXCEPT items #7 and #8 which are reversed scored. Score range 0-52. A score of less than 30 indicates severe fatigue. The higher the score, the better the quality of life.

http://www.facit.org/FACITOrg/Questionnaires

Two minute walk test instructions

Patients will need to walk for a total of two minutes and the unit of length will be measured in "feet." Assistive devices can be used but should be kept consistent and documented from test to test. If physical assistance is required, this test should not be performed. Walking path (i.e. laps up and down hallway) should be predetermined and feasible to measure.
* Adapted from NIH toolbox

30 second chair stand test[49]

Patients should start in the sitting position with back straight, arms crossed over chest and feet flat on the floor. The chair should be placed against a wall for stability. Observe 1-2 practice stands to ensure proper form and adequate balance. Once the patient is ready, measure the number of times he/she can rise to a full stand and then return to a fully seated position in 30 seconds. If the patient is more than half way up at the end of the 30 seconds, count this as a full stand.