NCI Protocol #: 10417
Version Date: March 11, 2025

SUMMARY OF CHANGES – protocol

To: CTEP Protocol and Information Office

From: Michael Savona, M.D.

Date: March 11, 2025

Re: Amendment to Protocol #10417: "Clinical Evaluation of ASTX727 in Combination with Venetoclax All-Oral Therapy vs Standard of Care Cytarabine and Anthracycline Induction Chemotherapy for Younger ELN High Risk FLT3^{WT} Patients with Acute Myeloid Leukemia." This protocol has been amended to decrease the time of the long-term follow-up given the early closure of the trial by CTEP in December 2023 due to low accrual.

SUMMARY OF CHANGES – Protocol

I. Comments Requiring a Response – Major Issues:

#	Section	Comments
1.	Stats overall	The amendment changes the primary endpoint from EFS to OR for this stopped early study with available almost two years of follow-up on the enrolled patients. Under the circumstance, there is no enhancement in the study results' interpretability from changing the primary endpoint. Instead, if the study team desires to report the study after certain follow-up period, the amendment should specify the desired reporting time without changes to the primary/secondary endpoints. **PI Response: We received this advice from CTEP personnel. Upon further discussion with personnel from CTEP, it became clear the prior communication did not have its intended meaning. Thus, we have removed the changes to the primary endpoints to retain the EFS. We have kept the alterations to the long-term follow-up period which covers the time served for the two patients who remained in LTFU when the study was closed.**
2.	Stats overall	There is no explanation for why the study stopped or why the changes proposed are being made. This needs to be clarified. **PI Response: We did not choose to stop this study as it was administratively closed to accrual on 12/18/23 by CTEP due to low accrual. As no meaningful analysis can be achieved from following the 1-2 patients who remain in LTFU before the trial was abruptly closed, we have decided to reduce the LTFU from 5 years to 2 years to decrease the burden of follow-up visits on the patients and staff.**

II. Comments Requiring a Response– Administrative & Editorial Issues:

#	Section	Comments
3.	10.1.1.2	Please update ASTX727 CAEPR to Version 2.2, December 19, 2024. **PI Response: With respect, we do not agree with the necessity to change an ICD for a trial that has not accrued any new patients and has not treated patients since 12/18/23.**
4.	10.3.3.	Note that the AE Reporting Table for Phase 1 and Early Phase 2 Studies provided in Section 10.3.3 of the protocol needs to be revised. Please update as per the current NCI Guidelines for Investigators, Effective Date: August 30, 2024. **PI Response: Respectfully, we do not see the necessity in modifying the protocol language to current NCI guidelines for a protocol that has not enrolled a new patient since December 2023 and will not be enrolling any new patients in the future.**
5.	ICD Phase 1b and Phase 2 Drug Risk	Please update the Possible Side Effects of ASTX727 with template language (CAEPR Version 2.2, December 19, 2024). **PI Response: With respect, we do not agree with the necessity to change an ICD for a trial that has not accrued any new patients and has not treated patients since 12/18/23.**

I. Changes initiated by the Principal Investigator:

#	Section	Comments
1.	Protocol_Type_Version	**PI Comment:** We have amended the amendment number to Amendment 6, Version 1.0, March 11, 2025.
2.	6.5	**PI Comment:** The duration of follow-up was reduced from 5 years to 2 years.
3.	9.1	**PI Comment:** We reduced the duration of follow-up from 5 years to 2 years.
4.	11.1	**PI Comment:** We changed the survival follow-up in footnote (t) from 5 years to 2 years.
5.	11.2	**PI Comment:** We changed the survival follow-up in footnote (q) from 5 years to 2 years.
6.	Global	**PI Comment:** We have updated the dates throughout to match the amended document version date.

NCI Protocol #: 10417

Local Protocol #: VICCNCIHEM10417

ClinicalTrials.gov Identifier: NCT04817241

TITLE: Clinical Evaluation of ASTX727 in Combination with Venetoclax All-Oral Therapy *vs.* Standard of Care Cytarabine and Anthracycline Induction Chemotherapy for Younger $FLT3^{WT}$ Patients with ELN High- Risk Acute Myeloid Leukemia

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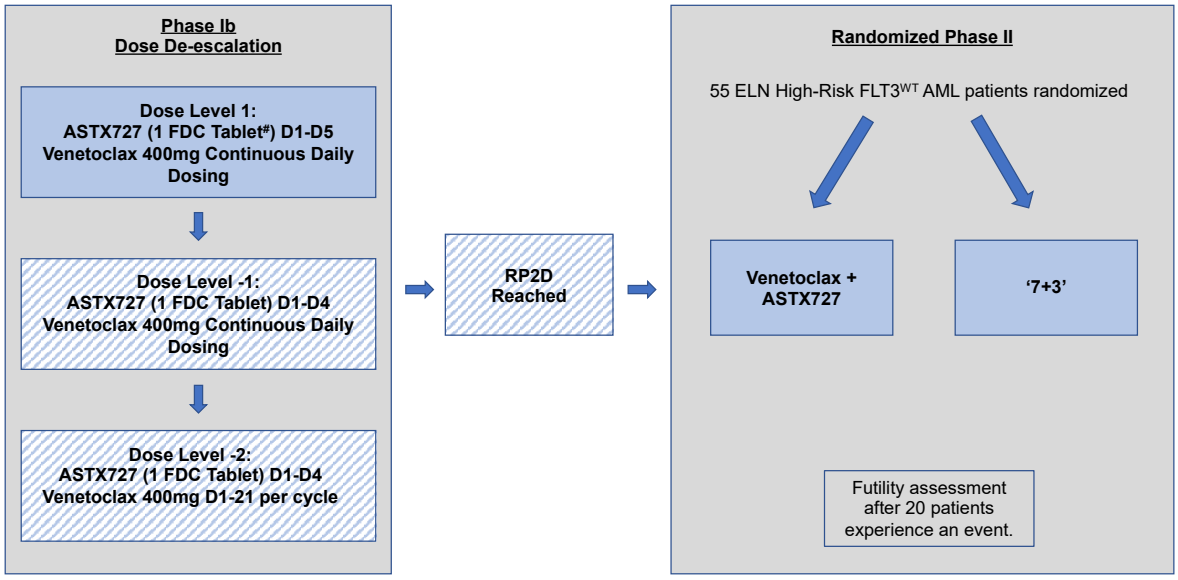
NCI-Supplied Agent(s): Venetoclax (NSC 766270) and ASTX727 (NSC 820631)
Other Agent(s): Daunorubicin (NSC 82151), commercial; and cytarabine (NSC 63878), commercial

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IND Sponsor: DCTD, NCI

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Original / November 3, 2020
Revision 1 / January 14, 2021
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Amendment 2 / Version 1.0 / September 8, 2021
Amendment 3 / Version 1.0 / May 20, 2022
Amendment 3 / Version 2.0 / October 19, 2022
Amendment 4 / Version 1.0 / July 13, 2022
Amendment 5 / Version 1.0 / November 15, 2022
Amendment 6 / Version 1.0 / March 11, 2025

SCHEMA

This is a phase 2 study of ASTX727 (an oral combination of cedazuridine and decitabine) plus venetoclax in patients 18-65 years old with *FLT3*^{WT} ELN High- Risk acute myeloid leukemia (AML). The phase 2 portion will be a randomized trial comparing the two study arms: the intervention arm *vs*. the standard of care treatment (daunorubicin plus cytarabine '7+3') as the control arm. The primary objective will be to determine the preliminary efficacy of the intervention compared to the 7+3 treatment with a primary endpoint of event-free survival (EFS). (Note: The Phase 1b portion of the trial is now closed. Moving forward, all new patients will be enrolled in the Phase 2 portion of the trial as per Amendment #04 (July 13, 2022).



#ASTX727 is a Fixed Dose Combination (FDC) tablet containing 35mg decitabine + 100mg cedazuridine

Dose schedule for phase 1b			
Dose Level	**Dose**		**Cycle Length**
	ASTX727 (cedazuridine/decitabine), PO	**Venetoclax, PO****	
*Level 1	1FDC Tablet[#], QD D1-5	400mg, QD D1-28	28 days
Level -1	1FDC Tablet, QD D1-4	400mg, QD D1-28	
Level -2	1 FDC Tablet, QD D1-4	400mg, QD D1-21	
*Starting dose level [#]ASTX727 is a Fixed Dose Combination (FDC) tablet containing 35mg decitabine + 100mg cedazuridine **Follow ramp up dosing scheme in Section 6.1.1.2. PO = Per Oral, QD = Once a Day			

Phase 2 – Venetoclax + ASTX727 Arm

Agent	Dose	Route	Schedule	Cycle Length
Venetoclax	400mg	PO	Continuous daily	28 days
ASTX727	1 FDC Tablet[#]	PO	Days 1-5	28 days
[#]ASTX727 is a Fixed Dose Combination (FDC) tablet containing 35mg decitabine + 100mg cedazuridine PO = Per Oral				

Phase 2 – 7+3 Arm

Agent	Dose	Route	Schedule	Cycle Length
Cytarabine	100 mg/m^2	IV	Days 1-7 continuous infusion	28 days
Daunorubicin	60 mg/m^2	IV	Days 1, 2, 3	28 days
IV = Intravenous				

TABLE OF CONTENTS

SCHEMA 5

1. OBJECTIVES 10
   - 1.1 Primary Objectives.....10
   - 1.2 Secondary Objectives.....10
   - 1.3 Exploratory Objectives 10

2. BACKGROUND 10
   - 2.1 Study Disease(s).....11
   - 2.2 CTEP Agents 11
   - 2.3 Other Agent(s) 21
   - 2.4 Rationale 22
   - 2.5 Correlative Studies Background 28

3. PATIENT SELECTION 37
   - 3.1 Eligibility Criteria 37
   - 3.2 Exclusion Criteria 38
   - 3.3 Inclusion of Women and Minorities 39

4. REGISTRATION PROCEDURES 40
   - 4.1 Investigator and Research Associate Registration with CTEP 40
   - 4.2 Site Registration.....41
   - 4.3 Patient Registration.....43
   - 4.4 General Guidelines.....45

5. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES 45
   - 5.1 Summary Table for Specimen Collection.....45
   - 5.2 Specimen Procurement Kits and Scheduling.....46
   - 5.3 Specimen Tracking System Instructions.....46
   - 5.4 Specimen Collection 50
   - 5.5 Shipping Specimens from Clinical Site to the EET Biobank – Leukemia Division.....51
   - 5.6 Biomarker Plan 53
   - 5.7 Integrated Correlative Studies.....56
   - 5.8 Exploratory/Ancillary Correlative Studies 57

6. Treatment plan 59
   - 6.1 Agent Administration.....59
   - 6.2 Definition of Dose-Limiting Toxicity.....70
   - 6.3 General Concomitant Medication and Supportive Care Guidelines.....72
   - 6.4 Duration of Therapy.....75
   - 6.5 Duration of Follow-Up 76

7. DOSING DELAYS/DOSE MODIFICATIONS 76
   - 7.1 CTEP Agents 76

7.2 Commercial Agents 79

8. PHARMACEUTICAL INFORMATION....80
8.1 CTEP IND Agents 80
8.2 Commercial Agents 86

9. STATISTICAL CONSIDERATIONS....89
9.1 Study Design/Endpoints....89
9.2 Sample Size/Accrual Rate....90
9.3 Analysis of Secondary Endpoints 90
9.4 Reporting and Exclusions 93
9.5 Data Safety Monitoring Board....93

10. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS 94
10.1 Comprehensive Adverse Events and Potential Risks Lists (CAEPRs) 94
10.2 Adverse Event Characteristics 99
10.3 Expedited Adverse Event Reporting....99
10.4 Routine Adverse Event Reporting 103
10.5 Pregnancy....103
10.6 Secondary Malignancy....103
10.7 Second Malignancy....103

11. STUDY CALENDAR 104
11.1 Study Calendar for Venetoclax+ASTX727 Combination 105
11.2 Study Calendar for 7+3 Control Treatment Arm....109

12. MEASUREMENT OF EFFECT....112
12.1 Definitions....112
12.2 Antitumor Effect – Hematologic Tumors....112
12.3 Exploratory Antitumor Effect – Hematologic Tumors....113

13. STUDY OVERSIGHT AND DATA REPORTING / REGULATORY REQUIREMENTS....113
13.1 Study Oversight 113
13.2 Data Reporting....114
13.3 Data Quality Portal 116
13.4 CTEP Multicenter Guidelines....116
13.5 Collaborative Agreements Language....117
13.6 Genomic Data Sharing Plan....118

14. REFERENCES 119

APPENDIX A 2017 ELN RECOMMENDATIONS: AML RISK....130

APPENDIX B PERFORMANCE STATUS CRITERIA 131

APPENDIX C FORMULA TO ESTIMATE RENAL FUNCTION USING

SERUM CREATININE....132

APPENDIX D: PATIENT CLINICAL TRIAL WALLET CARD....133

APPENDIX E PATIENT MEDICATION DIARY....134

APPENDIX F PATIENT MEDICATION DIARY FOR ANTIEMETIC PROPHYLAXIS FOR PATIENTS RECEIVING **Venetoclax and** ASTX727....137

APPENDIX G PATIENT MEDICATION DIARY FOR ANTIEMETIC PROPHYLAXIS FOR PATIENTS RECEIVING **7+3 Chemotherapy**....138

APPENDIX H BONE MARROW SAMPLE PROCESSING AND CRYOPRERVATION PROTOCOL....139

APPENDIX I Pharmacokinetic Study Sample Handling....142
1.1 Venetoclax Plasma Pharmacokinetic Samples....142
1.2 Handling of Specimens(s)....142
1.3 Shipping of Specimen(s)....143

1. OBJECTIVES

1.1 Primary Objectives

1.1.1 To determine and compare the preliminary efficacy of venetoclax +ASTX727 *vs.* standard anthracycline induction therapy ('7+3') with a primary endpoint of event-free survival (EFS).

1.2 Secondary Objectives

1.2.1.1 To determine the complete response (CR + CRi) rate in patients with treatment naïve *FLT3*^{WT} AML treated with venetoclax and ASTX727 *vs.* standard anthracycline induction therapy ('7+3').

1.2.1.2 To determine the duration of response (DoR) in patients with treatment naïve *FLT3*^{WT} AML treated with venetoclax and ASTX727 *vs.* standard anthracycline induction therapy ('7+3').

1.2.1.3 To determine the progression free survival (PFS) of patients with treatment naïve *FLT3*^{WT} AML treated with venetoclax and ASTX727 *vs.* standard anthracycline induction therapy ('7+3').

1.2.1.4 To determine the overall response rate (ORR) in patients with treatment naïve *FLT3*^{WT} AML treated with venetoclax and ASTX727 *vs.* standard anthracycline induction therapy ('7+3').

1.2.1.5 To determine the overall survival (OS) of patients with treatment naïve *FLT3*^{WT} AML treated with venetoclax and ASTX727 *vs.* standard anthracycline induction therapy ('7+3').

1.2.1.6 To determine the proportion of patients receiving stem cell transplantation (SCT) in patients with treatment naïve *FLT3*^{WT} AML treated with venetoclax and ASTX727 *vs.* standard anthracycline induction therapy ('7+3').

1.2.1.7 To identify mutational burdens in venetoclax +ASTX727 sensitive *vs.* resistant AML LICs.

1.3 Exploratory Objectives

1.3.1 To identify transcriptomic signatures in venetoclax +ASTX727 sensitive *vs.* resistant AML LICs.

1.3.2 Determine the utility of high-throughput phenotype-based assessment of drug efficacy for predicting patient response to venetoclax +ASTX727.

1.3.3 Determine if treatment failure is a function of therapy sequence or results in resistance to the alternative therapy by conducting a co-clinical trial via patient-derived xenograft (PDX).

1.3.4 To characterize the pharmacokinetics of venetoclax

1.3.5 To determine the morphologic leukemia-free state (MLFS) rate in patients with treatment naïve *FLT3*^{WT} AML treated with venetoclax and ASTX727 *vs.* 7+3 threrapy.

2. BACKGROUND

2.1 Study Disease(s)

2.1.1 Acute Myelogenous Leukemia

The American Cancer Society estimates 21,450 new diagnoses of AML in the United States in 2019 and an alarming 10,920 deaths of patients with this diagnosis (American Cancer Society, Key statistics acute myeloid leukemia (AML), 2019). The median age of diagnosis for AML is 68 years, and younger patients experience better disease-free survival than elderly patients (Dohner *et al.*, 2010). However, with the exception of patients with favorable risk PML-RARa re-arrangements, isolated *NPM1* mutation, or core binding factor abnormality [inv16/t(16;16) or t(8;21)], of which many can be cured with chemotherapy alone (Fernandez *et al.* 2009 and Lo-Coco *et al.*, 2013), the long term survival from AML is dismal. Large registry analyses have found that patients with intermediate or poor risk disease make up between 85-91% of all cases in younger patients, and these patients expect overall survival at 5 years to be between 3-40%, based on molecular or cytogenetic risk (Dombret *et al.*, 2015; Grimwade *et al.*, 2010; and Pautas *et al.*, 2010). Although there had been no major change in induction therapy strategy for nearly 4 decades (Yates *et al.*, 1973), 2017-18 saw the approval of several new breakthrough therapies for sub-groups of patients with AML (DiNardo *et al.,* 2019; DiNardo *et al.,* 2018a; DiNardo *et al.,* 2018b; Lancet *et al.,* 2018; Perl *et al.,* 2017; Savona *et al.,* 2018a; Stein *et al.,* 2017; and Stone *et al.,* 2017). Still, improvements in expected outcomes are incremental for most of these therapies, and with exception of targeted therapy directed at FMS-like tyrosine kinase (FLT3), none of these advances has yet to result in clear, meaningful long term improvements in survival for younger patients (DiNardo *et al.,* 2018a; DiNardo *et al.,* 2018b; Lancet *et al.,* 2018; Savona *et al.,* 2018a; and Stein *et al.,* 2017).

2.2 CTEP Agents

2.2.1 Venetoclax

B-cell lymphoma 2 (BCL2) is a mitochondrial pathway anti-apoptosis protein that plays a key role in survival and persistence of AML blasts (Vo *et al.*, 2012; Konopleva *et al.*, 2006). BCL2 overexpression has been implicated in chemotherapy resistance and inferior overall survival in AML (Konopleva *et al.*, 2006). Venetoclax (ABT-199/GDC-0199) is a potent and highly selective small molecule BCL2 inhibitor that has shown preclinical and clinical activity across a range of hematologic malignancies, including AML. Targeted BCL2 inhibition with venetoclax has shown to induce cell death in AML cell lines and leukemic blasts and stem cells while sparing normal hematopoietic stem cells. (Vo *et al.*, 2012; Konopleva *et al.*, 2006; Lagadinou *et al.*, 2013). In the relapsed-refractory (R/R) AML setting, a phase 2 study examined the single agent activity of venetoclax. The CR rate was 19% with another 19% having some form of myeloblast reduction. The drug was well tolerated with AEs consistent with expectations in the R/R AML population. Following its modest clinical activity and acceptable safety profile as monotherapy, venetoclax was investigated in phase 1 studies in combination with DNA methyltransferase inhibitors (DNMTi) decitabine or 5'azacitidine (azacitidine) (NCT02203773) (Konopleva *et al.*, 2016). This drug combination strategy is based on *ex vivo* pre-clinical evidence that combining venetoclax with azacitidine was potently synergistic in inducing AML cell death (Bogenberger *et al.*, 2015). Studies suggest that azacitidine downregulates anti-

apoptotic Mcl-1 protein concentrations, which is a known mediator of resistance to venetoclax (Tsao *et al.*, 2012). Furthermore, the combination was shown to induce apoptosis in a p53 independent manner which has potential therapeutic implications in mutant *TP53* AML (Tsao *et al.*, 2012).

2.2.1.1 Summary of Nonclinical Studies

2.2.1.1.1 Nonclinical Pharmacology

In vitro, venetoclax demonstrated cell killing activity against patient-derived chronic lymphocytic leukemia (CLL) and AML cells and a variety of lymphoma and leukemia cell lines, including B-cell follicular lymphomas (FLs), mantle cell lymphomas (MCLs), diffuse large B-cell lymphomas (DLBCLs), AMLs, and multiple myeloma (MM) cell lines (Venetoclax Investigator's Brochure, 2020). Venetoclax was especially potent against non-Hodgkin lymphoma (NHL) cell lines expressing high levels of BCL2. Venetoclax inhibits subcutaneous xenograft growth of human tumor cell lines derived from acute lymphoblastic leukemia (ALL), NHL, and AML, and is highly efficacious using various doses and combined with other regimens. The drug is also active in a model of disseminated ALL and AML. Venetoclax enhanced the activity of a broad variety of chemotherapeutic agents in other human hematological models. Specifically, venetoclax enhances the efficacy of bendamustine plus rituximab (BR) in models of mantle cell lymphoma (MCL) and DLBCL. Furthermore, venetoclax demonstrated potential to enhance the efficacy of bortezomib in multiple models of MM.

2.2.1.1.2 Nonclinical Safety Pharmacology

Venetoclax was tested in a battery of safety pharmacology assays and produced no effects in central nervous system (CNS)/neurobehavioral, or respiratory studies in mice at oral doses up to 600 mg/kg (Venetoclax Investigator's Brochure, 2020). In dogs, mild reductions in cardiac contractility and cardiac output were observed at plasma concentrations of ≥ 16 µg/mL; concentrations greater than the concentration of venetoclax in humans (average maximum observed plasma concentration [C_{max}] = 3.46 µg/mL at the 1200 mg dose). However, no effects on blood pressure, heart rate, or electrocardiogram (ECG) parameters were observed in dogs at a maximum drug concentration of 46 µg/mL.

2.2.1.1.3 Nonclinical Product Metabolism

In rats, venetoclax was widely distributed into liver, kidneys, spleen, heart, lungs, small intestine, and white fat, but was poorly distributed in testes, brain, muscle, bone, and pigmented tissues (Venetoclax Investigator's Brochure, 2020). Biotransformation of venetoclax proceeded via a combination of oxidation and conjugation. Venetoclax and M27 (major human metabolite) are predominately metabolized by cytochrome P450 (CYP) 3A4 *in vitro*. Clinical studies have supported the *in vitro* observations for venetoclax as a sensitive substrate of CYP3A4. At the 400 mg once daily (QD) dose venetoclax and M27 are not predicted to be perpetrators of the major CYP enzymes, but venetoclax may weakly inhibit UGT1A1. Both venetoclax and M27 are substrates for the efflux transporters P-glycoprotein (P-gp) and breast cancer resistance

protein (BCRP), and inhibitors or inducers of these transporters are expected to cause change in the exposure of venetoclax and M27. Venetoclax and M27 are P-gp and BCRP inhibitors and may interact with substrates for these transporters. Venetoclax may inhibit organic anion transporter protein 1B1 (OATP1B1) and cause weak interaction with drugs that are substrates of this transporter.

2.2.1.1.4 Nonclinical Toxicology

The primary toxicities associated with venetoclax were effects on the hematologic system (decreased lymphocytes and erythrocytes), the male dog reproductive system (testicular germ cell depletion), and embryofetal toxicity in mice (Venetoclax Investigator's Brochure, 2020). Other noteworthy but non-adverse findings were minimal to mild single cell necrosis in epithelial tissues (gallbladder, exocrine pancreas, epididymides, prostate, and stomach) of dogs and hair coat color change towards white in mice and dogs that was a likely result of loss of melanin pigment in the hair. These effects of venetoclax were generally consistent with pharmacologic mechanism of action (selective BCL2 inhibition) of venetoclax.

2.2.1.2 Summary of Clinical Studies

As of 28 November 2019, on the basis of data available in the clinical databases for company-sponsored studies with unblinded data, a total of 3974 subjects have been exposed to at least 1 dose of venetoclax across company-sponsored studies (Venetoclax Investigator's Brochure, 2020). Of these subjects, 3962 were included in the overall pooled analyses for reporting Reference Safety Information (RSI): 3610 oncology subjects, 255 healthy volunteers, 24 subjects with hepatic impairment, and 73 SLE subjects. Of the 3610 oncology subjects, 3481 were adults in the venetoclax oncology program (1183 in monotherapy studies and 2298 in combination therapy studies): 1639 with CLL/SLL, 597 with AML, 483 with MM, 572 with NHL, 144 with myelodysplastic syndrome (MDS), and 44 with ALL/LL, 1 with rhabdomyocarcoma, and 1 with Evans tumor. The remaining oncology subjects included 65 pediatric subjects in the venetoclax program and 64 oncology subjects in studies conducted with other investigational new drug (IND) compounds outside the venetoclax program. An additional 452 subjects with blinded data have been treated with either venetoclax combination therapy or a comparator treatment in company-sponsored venetoclax oncology studies.

2.2.1.2.1 Clinical Pharmacokinetics

Pharmacokinetic data for venetoclax are available from studies in subjects with cancer (CLL/SLL, AML, ALL, lymphoblastic lymphoma [LL], NHL, and MM), healthy subjects, and subjects with hepatic impairment, and pediatric subjects (Venetoclax Investigator's Brochure, 2020). Following multiple-dose administration, the maximum plasma concentration of venetoclax was attained 5 to 8 hours after dosing. The harmonic mean terminal phase elimination half-life ($t_{1/2}$) ranged from 17 to 41 hours following a single oral dose of venetoclax. In subjects with CLL, venetoclax showed minimal accumulation, and steady-state AUC increased proportionally over the dose range of 150 to 800 mg. Venetoclax has been administered with food in all clinical studies, as food increased the bioavailability of venetoclax by approximately 3- to 5-fold. Venetoclax is highly bound to plasma proteins with unbound

fraction (fu) < 0.01, and it is primarily eliminated as metabolites in feces with negligible renal elimination (< 0.1%). Based on the population pharmacokinetic analysis, age, sex, race, weight, and mild and moderate renal or hepatic impairment do not have an effect on venetoclax clearance. Venetoclax is associated wide inter-individual pharmarcokinetic variability (%CV in apparent oral clearance of ~50%), and overlap in exposure over a 5-fold dose range (Davids *et al*., 2017). Venetoclax wide inter-individual pharmacokinetic variability is likely due to high drug-drug interaction (DDI) potential. Venetoclax is metabolized by CYP3A4/5 and is a substrate for the efflux transporters P-glycoprotein (P-gp) and BCRP, and is possibly a substrate for the liver uptake transporter OATP1B1. To evaluate DDI potential with venetoclax as victim drug, pharmacokentic studies have shown that administration of ketoconazole and posaconazole, strong inhibitors of CYP3A and OATP1B1 as well as inhibitors of P-gp and BCRP, increased venetoclax AUC by 6.4-fold and 9-fold, respectively (Agarwal et al., 2017; Freise et al., 2018). In addition, 14 days of treatment with ritonavir, a strong CYP3A4, P- gp and OATP1B1 inhibitor, increased venetoclax AUC by 690%; and a single-dose of rifampin, a P-gp and OATP1B1 inhibitor, increased venetoclax exposure by 78%.

2.2.1.2.2 Clinical Safety and Efficacy

The important identified risks for venetoclax are tumor lysis syndrome (TLS), neutropenia, and serious infection (Venetoclax Investigator's Brochure, 2020). Tumor lysis syndrome is a risk associated with venetoclax treatment and it is highest in CLL and MCL. Other adverse events commonly observed with venetoclax include nausea, diarrhea, and other hematological effects (including, anemia, thrombocytopenia, and lymphopenia). Events of anemia, neutropenia, and thrombocytopenia are also commonly observed with the underlying hematologic malignancies. Serious infections, including events of sepsis and events with fatal outcome, have been reported with venetoclax treatment and their incidence is higher with combination treatments. Co-administration with CYP3A inducers and inhibitors can cause changes in venetoclax exposure. Events of second primary malignancies (including non-melanoma skin cancers) have been reported across the oncology program and no pattern has been observed. Second primary malignancies are monitored across the development program as venetoclax is being evaluated in many indications where subjects have R/R disease that had previously been treated with various cytotoxic agents. Richter's transformation cases have been observed in patients with CLL treated with venetoclax and the rate is within the range of reported incidence for patients with CLL.

Venetoclax was recently approved in combination with decitabine or azacitidine or low dose cytarabine (LDAC) in untreated older patients with AML. Given the low toxicity and high response rate, venetoclax -based regimens represent an emerging treatment paradigm in AML; in these studies, 50-70% of patients deemed unfit to receive cytotoxic induction chemotherapy achieved complete remission (CR) or CR with incomplete recovery (CRi) (DiNardo *et al.*, 2019; Wei *et al.*, 2019). Younger AML patients (age 18-65 years) typically receive cytotoxic chemotherapy that yields complete responses (CRs) that range from 30-70% of patients with an associated treatment-related mortality of 3-5% with considerable morbidity. Many of these who respond exceptionally to intensive cytotoxic chemotherapy are considered favorable risk by NCCN and ELN guidelines by virtue of specific cytogenetic abnormalities (e.g. core binding factor leukemia). The remainder of the patients with intermediate risk and unfavorable risk AML (approximately 90% of young AML), have much lower response rates and unacceptable 5-

year survival (3-40%; as low as 3-5%) (Dombret *et al.*, 2015; Grimwade *et al.*, 2010; Pautas *et al.*, 2010). Therefore, there is an urgent need for more effective, safer, and practical treatments for AML in younger patients. There is no known age-bias with respect to the mechanism of action with venetoclax, and preclinical evaluation indicates considerable responses in samples from younger AML patients (Pollyea *et al.*, 2018; Ramsey *et al.*, 2018), yet efficacy of venetoclax-based therapy has yet to be reported in a younger, healthier AML patient population, or compared to standard of care. Single arm studies of venetoclax + azacitidine (NCT03573024) or venetoclax + decitabine (NCTT03844815) are currently underway, which include younger patients, and plans for studies randomizing venetoclax +DNMTi to anthracycline induction in younger patients, similar to the design in this proposal, are now being made by others.

2.2.2 ASTX727

ASTX727 is the oral fixed-dose combination (FDC) of E7727 (cedazuridine) + decitabine (ASTX727 Investigator's Brochure, 2020). Cedazuridine is a cytidine deaminase (CDA) inhibitor; decitabine is a nucleoside hypomethylating agent (HMA) known to induce demethylation through deoxyribonucleic acid (DNA) methyltransferase inhibition. Decitabine is a substrate of CDA. The combination of cedazuridine + decitabine has been shown to increase systemic decitabine exposure (area under the curve [AUC]) following oral administration.

2.2.2.1 Summary of Nonclinical Studies

Information in this section pertains to E7727 (cedazuridine) or the combination of cedazuridine and decitabine. Refer to the current United States Dacogen® package insert (USPI) for additional nonclinical information regarding decitabine.

2.2.2.1.1 Nonclinical Pharmacology

In vivo studies in mouse leukemia models showed that administration of oral decitabine after oral cedazuridine was efficacious, whereas cedazuridine administered alone has no anticancer activity (ASTX727 Investigator's Brochure, 2020). Plasma cytidine levels appeared to increase transiently in a dose-dependent manner in cynomolgus monkeys after treatment with cedazuridine. Pronounced long interspersed nucleotide elements (LINE-1) demethylation was induced in cynomolgus monkeys after oral co-administration of cedazuridine and decitabine.

2.2.2.1.2 Nonclinical Safety Pharmacology

Cedazuridine showed:

- Negligible inhibition of human ether-à-go-go-related gene (hERG) potassium current in human embryonic renal cells
- No effect on action potential parameters in guinea pig papillary muscle.
- No significant inhibition of binding in a receptor panel.
- No findings *in vivo* in cardiovascular study in telemetered monkeys at doses up to 200 mg/kg or *in vivo* in a central nervous system (CNS) safety pharmacology study in mice at doses up to 2000 mg/kg (ASTX727 Investigator's Brochure, 2020).

2.2.2.1.3 Nonclinical Pharmacokinetics

The cedazuridine pharmacokinetic (PK) profile is characterized by fast-to-moderate absorption, low volume of distribution, low clearance, and moderate elimination half-life (ASTX727 Investigator's Brochure, 2020).

- Cedazuridine co-administered with decitabine, with or without a delay, increased bioavailability of decitabine.
- PK exposures for oral decitabine increased when decitabine was dosed after cedazuridine in mice and monkeys.
- In mass balance in monkeys, cedazuridine was excreted mainly via the kidneys after intravenous (IV) dose.
- Cedazuridine and decitabine do not inhibit nor induce human CYP enzymes and are not a substrate or inhibitor of P-gp or other major human drug transporters.
- Cedazuridine did not inhibit major human drug transporters when tested in interaction studies *in vitro*.
- Human metabolites were identified in clinical plasma samples, with the cedazurudine-epimer as the only major (>10% parent) entity. Toxicological coverage for metabolites is available as all metabolites detected in vivo in humans were also present in mouse and monkey plasma.

2.2.2.2 Summary of Clinical Studies

As of the data cutoff date (16 January 2020), a total of 309 subjects have received any dose of cedazuridine (ASTX727 Investigator's Brochure, 2020). Of these 309, 274 subjects with higher risk MDS (Int-1 to high-risk MDS per IPSS, including CMML) have been treated with cedazuridine and decitabine administered concomitantly as separate capsules or the ASTX727 FDC tablet (100 mg cedazuridine and 35 mg decitabine). Eight healthy subjects received only cedazuridine in the completed ADME study E7727-01. The remaining 27 subjects with lower risk MDS (low-risk or Int-1 per IPSS) received 100 mg cedazuridine and lower doses of decitabine (≤20 mg) in the low-dose ASTX727 clinical program.

2.2.2.2.1 Clinical Pharmacokinetics and Pharmacodynamics

Study ASTX727-01 (ASTX727 Investigator's Brochure, 2020):

- Oral administration of ASTX727 (100 mg cedazuridine and 35 mg decitabine) as separate capsules or as the FDC tablet achieved the primary study objective, showing that mean decitabine 5-day $AUC_{0\text{-}t}$ was equivalent to IV decitabine 20 mg/m^2, based on the predefined equivalence limits of AUC in the sample size assumptions for each stage.
- Oral administration of ASTX727 achieved LINE-1 DNA demethylation similar to IV decitabine at 20 mg/m^2.

Study ASTX727-02 (ASTX727 Investigator's Brochure, 2020):

- The primary analysis of the primary study endpoint showed that the 5-day $AUC_{0\text{-}24h}$ ratio of geometric LSM for ASTX727 (oral) relative to IV decitabine (IV) was 98.93% (90% CI: 92.66, 105.6). The two-sided 90% CI is contained entirely within the prespecified range of 0.80 to 1.25 for the primary analysis.

- In the treatment comparisons in Cycle 1 and Cycle 2, the difference in maximum %LINE-1 demethylation between IV decitabine and ASTX727 was <1%, consistent with similar biological and PD effects between the 2 treatments.

Study ASTX727-04 (ASTX727 Investigator's Brochure, 2020):

- Equivalence analysis for plasma decitabine PK parameters showed lower exposures following fed conditions compared with fasted conditions. Decitabine total exposures (AUC) decreased by approximately 40%, and peak exposures (C_{max}) decreased by approximately 53.5% when ASTX727 was taken with food. The reduction in decitabine systemic exposures was considered clinically meaningful due to the magnitude of the effect.
- Unlike decitabine, changes in cedazuridine or cedazuridine-epimer systemic exposures under fed conditions were not clinically meaningful as the magnitude was approximately 10-20%. Cedazuridine total (AUC) and peak (C_{max}) exposure were approximately 10% higher following high-fat breakfast compared to those following fasted conditions, tmax was slightly longer with food, which might contribute to the decrease in decitabine exposure observed with food.

Study E7727-01 (14C-cedazuridine ADME in healthy volunteers) (ASTX727 Investigator's Brochure, 2020):

- After oral administration, the mean fraction absorbed of cedazuridine was 47.5%, with a geometric mean absolute bioavailability of 20.7%.
- A possible minor association of [14C]-radioactivity with red blood cells (RBCs) was observed over time.
- The ratio of cedazuridine to cedazuridine-epimer was higher after IV administration than after oral administration, indicating that most of the conversion of cedazuridine to cedazuridine-epimer occurs presystemically (prior to absorption). Cedazuridine and cedazuridine-epimer accounted for the majority of [14C] radioactivity in plasma, with only 1 other metabolite (dehydrogenation) identified in plasma. This metabolite and 2 urea conjugates were identified in urine as well as cedazuridine and cedazuridine-epimer.
- Cedazuridine was primarily eliminated renally, with 80.9% of an IV dose recovered in urine within 120 hours of administration. The majority of the orally absorbed (fraction absorbed of 47.5%) dose was excreted in the urine. Unchanged cedazuridine and cedazuridine-epimer were both excreted in the urine (17.1% and 17.5% of the dose, respectively, after oral administration). The $t_{1/2}$ was similar for cedazuridine and cedazuridine-epimer and was slightly longer for total radioactivity.

2.2.2.2.2 Clinical Efficacy

Study ASTX727-01 Phase 1 Dose Escalation (ASTX727 Investigator's Brochure, 2020):

- Overall response rate: Analysis of all subjects (all cohorts combined) showed a complete response (CR) rate of 11% and an overall response rate of 30% (overall response = CR + partial response [PR] + marrow complete response [mCR] + hematological improvement [HI]).
- Duration of response: The mean duration of complete response was 355 days.

- Rate of transfusion independence: Of the 23 subjects who were RBC transfusion dependent at baseline (ie, who received 2 or more transfusions within 56 days of the first dose of study treatment), 7 (30%) were RBC transfusion free for any 56-day period post baseline. Of the 6 subjects who were platelet transfusion dependent at baseline, 2 (33%) were platelet transfusion free for any 56-day period post baseline.
- Time to Acute Myeloid Leukemia (AML) or death: Of the 44 total subjects, 13 (30%) reached the event (AML or death), and data were censored for the remaining 31 subjects (71%). Median time to AML or death was not reached.
- Overall survival: Of the 44 total subjects, 37 (84%) were alive as of the data cutoff date (01 June 2017); 7 subjects (16%) had died. Median survival time was not reached.

Study ASTX727-01 Phase 2 Overall (Dose Confirmation and FDC Stage Combined, N=80) (ASTX727 Investigator's Brochure, 2020):

- Best response rate: Results showed a CR rate of 18% (14/80), a PR rate of 0, and an mCR rate of 22.5% (18/80). A total of 16.3% of subjects (13/80) showed HI in 1 or more lineages. Overall response rate (CR+PR+mCR+HI) was 60% (48/80). A total of 40% of subjects (32/80) showed no response.
- Duration of response: Median duration of response for subjects with CR was 399 days. Median duration of response for subjects with a best response of mCR was 397 days.
- Rate of Transfusion Independence: Of the 38 subjects who were RBC transfusion dependent at baseline (ie, who received 2 or more transfusions within 56 days of the first dose of study treatment), 19/38 (50%) were RBC transfusion free for any 56-day period post baseline. Of the 12 subjects who were platelet transfusion dependent at baseline, 6/12 (50%) were platelet transfusion free for any 56-day period post baseline.
- Time to AML or Death: Of the 80 Phase 2 subjects, 47 (58.8%) reached the event (AML or death), and data were censored for the remaining 33 subjects (41.3%). Median time to AML or death was 364 days (12 months).
- Overall survival: Of the 80 Phase 2 subjects, 40 (50%) were alive as of the data cutoff date (05 June 2018); 40 subjects had died. Median survival time was 549 days (18 months).

Study ASTX727-02 Phase 3 Preliminary Results (ASTX727 Investigator's Brochure, 2020):

- Best Response Rate: Preliminary response results were evaluated for 3 groups of subjects: all treated subjects, treated subjects evaluable for response, and treated subjects with ≥6 months of follow up or who died or who discontinued treatment before 6 months. CR was observed in 9% to 13.4%, with an additional 28.4% to 45.5% achieving mCR. The overall response rate (CR+PR+mCR+HI) ranged from 44.8% to 64.4%. mCR with HI was reported in 10.5% to 13.9% of subjects.
- Rate of Transfusion Independence: One-third (32.7%) of the 52 subjects who were RBC transfusion dependent at baseline were RBC transfusion independent for any consecutive ≥56-day period post-baseline. A similar overall rate of platelet transfusion independence (30%) for any consecutive ≥56-day period post-baseline was observed for subjects with platelet transfusion dependence at baseline.

2.2.2.2.3 Clinical Safety

Study ASTX727-01 Phase 1 Dose Escalation (ASTX727 Investigator's Brochure, 2020):

- Subjects received a median of 5 cycles of treatment (range 1 to 35) and median follow up was 710.5 days (23 months).
- The adverse event (AE) profile observed in ASTX727-01 was consistent with that for IV decitabine administered at 20 mg/m^2 in the same schedule. The most frequently reported AEs Grade ≥3 were thrombocytopenia, fatigue, and constipation.
- Myelosuppression with associated infections remain the most prevalent AEs in this patient population; the most frequently reported SAEs were febrile neutropenia and pneumonia.

Study ASTX727-01 Phase 2 Overall (ASTX727 Investigator's Brochure, 2020):

- Subjects received a median of 7 cycles of treatment (range 1 to 29), and median follow up was 729 days (24 months).
- The incidence of AEs in Cycles 1 and 2 (including AEs Grade ≥3) was similar between IV decitabine and ASTX727 (as cedazuridine + decitabine capsules or as the FDC tablet).
- The most common AEs Grade ≥3 were neutropenia, thrombocytopenia, febrile neutropenia, leukopenia, and anemia. Cytopenias were generally similar between oral and IV treatments.
- SAEs reported with highest incidence were febrile neutropenia, sepsis, and pneumonia.
- No notable increase in GI events was observed with oral dosing. No safety signals were observed that could be attributed to the CDA inhibitor cedazuridine.
- The incidence of dyspnea was higher in the oral cycle than the IV cycle. Most dyspnea events were considered not related to study treatment.
- As of the CSR data cutoff date, 50% of subjects had died; 11 had treatment-emergent adverse events (TEAEs) with an outcome of death. All but one of these TEAEs (an event of myocarditis) were considered by the investigator to be not related to study treatment.

Study ASTX727-02 Phase 3 (ASTX727 Investigator's Brochure, 2020):

- Of the 133 subjects dosed, 129 (97%) received at least 2 cycles of treatment. As of the CSR data cutoff date, subjects had received a median of 4 cycles (range, 1 to 11), and 4 subjects (3%) had received 11 or more cycles.
- Overall, the most common AEs during treatment with ASTX727 were thrombocytopenia, neutropenia, anemia, fatigue, constipation, and nausea. The incidences of thrombocytopenia, neutropenia, anemia, leukopenia, febrile neutropenia, diarrhea, fatigue, and contusion were generally higher during treatment with ASTX727 than with IV decitabine in Cycles 1 or 2 but generally lower in ASTX727 Cycles ≥3 compared with IV decitabine.
- AEs Grades ≥3 that occurred with highest incidence during treatment with ASTX727 were thrombocytopenia, neutropenia, anemia, febrile neutropenia, and leukopenia. Overall, the incidence of AEs Grade ≥3 was generally similar or higher with ASTX727 treatment in the first 2 cycles compared with IV decitabine but generally similar or lower for ASTX727 in Cycles ≥3 compared with IV decitabine.
- The most commonly reported SAEs were febrile neutropenia, pneumonia, and

sepsis/septic shock. A higher incidence of SAEs was observed in ASTX727 Cycle 1 or 2 compared with IV decitabine Cycle 1 or 2; however, the incidence generally decreased with repeated ASTX727 cycle exposures (≥3 cycles).
- Five subjects who received ASTX727 had fatal TEAEs, including sepsis (2 subjects), pneumonia, cerebral hemorrhage, and septic shock (reported in 1 subject each).

Study ASTX727-03 (low dose) (ASTX727 Investigator's Brochure, 2020):
- A total of 23 subjects have been treated in this study as of the data cutoff date for the IB (16 Jan 2020). Enrollment is ongoing.
- The most frequently reported (≥20% of subjects) all-causality TEAEs through the data cutoff date were neutropenia, fatigue, and febrile neutropenia.
- The most frequently reported Grade 3 or Grade 4 TEAEs (≥10% of subjects) included neutropenia, febrile neutropenia, and anemia.
- A total of 10/23 subjects (43.5%) had an SAE; The most frequently reported (≥10% of subjects) SAE was febrile neutropenia.
- No subjects have experienced a fatal TEAE.

Study 393-102-0002 (low dose) (Japan) (ASTX727 Investigator's Brochure, 2020)
- Four subjects have been enrolled and treated as of the data cutoff date for the IB (16 Jan 2020). Enrollment is ongoing.
- All-causality TEAEs reported in 2 or more subjects through the data cutoff date were thrombocytopenia, constipation, anemia, leukopenia, and febrile neutropenia.
- All 4 treated subjects have experienced at least 1 Grade 3 TEAE as of the data cutoff date; Grade 3 events reported in 2 or more subjects were anemia, febrile neutropenia, and thrombocytopenia. Three subjects reported Grade 4 TEAEs, which included leukopenia (2 subjects), thrombocytopenia (2 subjects), and neutropenia (1 subject).
- SAEs were reported in 3 subjects: acute cholangitis, obstructive jaundice, and neutropenia in 1 subject; febrile neutropenia in 1 subject; and brain hemorrhage in 1 subject.
- No subjects have had a fatal TEAE as of the data cutoff date.

Study ASTX727-04 (food effect) (ASTX727 Investigator's Brochure, 2020):
- The most frequently reported AEs were neutropenia, anemia, and thrombocytopenia.
- The most frequently reported Grade ≥3 AEs were neutropenia, thrombocytopenia, anemia, and leukopenia.
- A total of 6 subjects (35.3%) experienced SAEs on study. SAEs that occurred in more than 1 subject included anemia and febrile neutropenia (each in 2 subjects, 11.8%).
- One of the 17 treated subjects (5.9%) died while on study treatment, due to atrial fibrillation that was not considered related to treatment.

Study ASTX727-06 (extension study) (ASTX727 Investigator's Brochure, 2020):
- A total of 16 subjects have been enrolled in ASTX727-06 from other clinical studies of ASTX727, and 9 of the 16 subjects were treated in the extension study as of the data cutoff date for the IB (16 Jan 2020). Enrollment is ongoing.
- All-causality TEAEs reported in treated subjects were thrombocytopenia, cholecystitis,

decreased neutrophil count, and dizziness (1 subject each).
- No subjects have experienced Grade 3 TEAEs through the data cutoff date; 2/16 subjects had Grade 4 TEAEs (thrombocytopenia and decreased neutrophil count).
- A total of 2/16 subjects (12.5%) had an SAE. SAEs reported in treated subjects were thrombocytopenia and cholecystitis.
- No subjects have had a fatal TEAE.

Study E7727-01 (14C-cedazuridine ADME) (ASTX727 Investigator's Brochure, 2020):
- Overall, cedazuridine was well tolerated. Six TEAEs were reported by 5 subjects (63%), including 2 events of nasopharyngitis, and 1 event each of anal hemorrhage, headache, diarrhea, and back pain.
- None of the AEs was considered related to treatment and all were of mild severity except the event of back pain, which was of moderate severity.
- No deaths, treatment discontinuation due to AEs, or SAEs occurred in the study.

The new oral therapy—ASTX727, which combines decitabine and cytidine deaminase inhibitor (CDAi) cedazuridine in a fixed dose combination—approximates the bioavailability and activity achieved with DEC (Savona *et al.*, 2019), and there is considerable appeal of substituting ASTX727 as an oral DNMTi into the venetoclax + regimen. The demands on patients for five consecutive days of parenteral dosing of DNMTi's are not trivial, and when oral *vs.* intravenous treatment strategies have been assessed, patients strongly prefer oral agents as long as efficacy is not significantly compromised. The goal of this study is to test the safety and preliminary efficacy of a 'patient-friendly' administration of venetoclax+ASTX727 in patients ages 18-65 years.

2.3 Other Agent(s)

2.3.1 "7+3" induction chemotherapy

The most commonly used induction regimen for AML is the so-called "7+3" regimen, which has been reviewed in detail (Dohner *et al.*, 2010). This regimen combines a seven-day continuous IV infusion of cytarabine (100 or 200 mg/m^2 per day) with a short infusion or bolus of an anthracycline given on days 1 through 3. The most commonly used anthracycline in this regimen is daunorubicin, but followed by idarubicin. The combination of "standard dose" cytarabine plus daunorubicin has been the historical standard for remission induction in AML. In this regimen, cytarabine is given by continuous IV infusion for 7 days plus daunorubicin by IV push or short infusion daily for the first three days. Standard dosing is: Cytarabine 100 mg/m^2 for 7 days plus daunorubicin 60mg/m^2 for 3 days.

2.3.1.1 Pharmacokinetics

Cytarabine is rapidly and widely distributed into tissues, including liver, plasma, and peripheral granulocytes. Rapid and extensive metabolism by cytidine deaminases occurs mainly in liver and kidneys. Cytarabine and its inactive metabolite are excreted in urine; 70-80% of cytarabine are eliminated in the urine within 24 hours (90% as inactive metabolite, 10% as intact drug). Initial cytarabine half-life elimination is 7 to 20 minutes; terminal cytarabine half-life elimination is 1 to 3 hours.

Daunorubicin distributes widely into tissues, particularly the liver, kidneys, lung, spleen, and heart; does not distribute into the central nervous system (CNS). Daunorubicin is metabolized primarily hepatically to daunorubicinol (active), then to inactive aglycones, conjugated sulfates, and glucuronides. It is excreted in feces (40%) and urine (~25% as unchanged drug and metabolites). Initial daunorubicin half-life elimination is 45 minutes; terminal cytarabine half-life elimination is 18.5 hours.

2.3.1.2 Efficacy

Depending on age and patient selection, as many as 80% of younger adults achieve a CR with these regimens. Most remissions happen after a single course. In the past, daunorubicin had been commonly employed at a dose of 45 mg/m^2; however, randomized studies suggest that higher doses of daunorubicin (*e.g.*, 60 to 90 mg/m^2 per day) are more effective and no more toxic than 45 mg/m^2. Overall survival of AML patients treated with daunorubicin 60 mg/m^2 or 90 mg/m^2 has not been compared and is often considered to be equivalent in standard practice.

2.3.1.3 Safety data

The combination of cytarabine plus daunorubicin results in severe pancytopenia in all patients and therefore requires transfusion support and antibiotics as needed. The median number of days with an absolute neutrophil count (ANC) less than 500 and a platelet count less than 50,000 are approximately 16 and 15, respectively. Common non-hematologic side effects seen in the majority of patients include stomatitis (mostly mild), alopecia, nausea, and vomiting (25% severe), and diarrhea (mostly mild to moderate). Cardiovascular side effects include cardiac failure (dose-related) and ECG abnormalities (transient; includes atrial premature contractions, S-T wave changes, supraventricular tachycardia, ventricular premature contractions; generally asymptomatic and self-limiting).

2.4 Rationale

2.4.1 Venetoclax +DNMTi therapy is an advance that should be tested in younger patients with high-risk AML.

ABT-199/venetoclax (venetoclax) is a potent, selective inhibitor of BCL2 that was evaluated as a single agent in relapsed/refractory AML (Lin *et al.*, 2016) and, ultimately, in combination with DNMTi azacytidine /decitabine and low-dose cytarabine (LDAC) as frontline therapy in patients with AML who were not candidates for induction chemotherapy (DiNardo *et al.*, 2019; DiNardo, Pratz et al, 2018; Wei et al, 2019). Despite advanced ages of patients in these studies, complete remission plus complete remission with incomplete count recovery (CR/CRi) rates of 67% were achieved in combination with DNMTi (DiNardo *et al.*, 2019; DiNardo, Pratz et al, 2018), and 54% with LDAC (Wei et al, 2019), far exceeding response rates reported for DNMTi or LDAC alone (Dombret *et al.*, 2015; Cashen *et al.*, 2010). Many responses occurred after just one cycle, with a low rate of early mortality, an absence of significant tumor lysis syndrome, and favorable tolerability. No patients younger than 65 years of age, however, were permitted on these trials of venetoclax +DNMTi/LDAC (DiNardo *et al.*, 2019; DiNardo, Pratz et al, 2018; Wei et al, 2019). The standard therapy for AML in patients under 65 years of age includes anthracycline and

cytarabine for those judged capable of safely receiving this therapy (Fernandez *et al.*, 2009; Yates *et al.*, 1973). Complete response rates from this high intensity induction strategy range from 25-55% for patients with intermediate and poor risk AML, and up to 30-40% of patients can be cured with allogeneic hematopoietic stem cell transplantation (allo-HSCT) (Dombret *et al.*, 2015; Grimwade *et al.*, 2010; Pautas *et al.*, 2010). Thus, alloHSCT is standard of care for eligible, consenting poor risk AML patients.

Mortality associated with the '7+3' induction treatment strategy is estimated to be 3-5% in addition to idiosyncratic organ toxicity experienced in 2-3% of fit patients and considerably more with addition of alloHSCT (Owattanapanich *et al.*, 2018). Further, many centers invoke a multiple induction treatment strategy at primary diagnosis, and patients who lose response are often re-induced with similar anthracycline+cytarabine regimens. In both cases, there is accelerating risk of treatment-associated toxicity (Rowe *et al.*, 2010). Still, improvements in supportive care (Kuderer *et al.*, 2006) in the setting of a lack of alternative options have long ensconced this paradigm into leukemia care despite poor long-term survival and toxicities. There has been no major change in induction therapy strategy for 40 years. Currently, there are only 2 studies registered at clinicaltrials.gov (NCT02993523 and NCT03941964) with venetoclax and DNMTi for younger patients, and neither of these trials randomize to standard of care '7+3', highlighting a novelty in this study.

2.4.2 ASTX727 is a DNMTi that approximates DEC and Provides Patients with an Oral Therapy Option



Figure 1: ASTX727 Phase 3 Study Design

Treatment with DNMTi consists of intravenous (IV) (DEC/AZA) or subcutaneous (SQ) (AZA) therapy for the first 5 (DEC/AZA) to 7 (AZA) days of every cycle (e.g. each cycle averages 28-42 days). The demands on patients for 5 consecutive days of a parenteral dosing strategy are not trivial. These patients experience pain associated with multiple IVs, or associated risks of infection, bleeding and thrombosis and inconveniences associated with central catheters or SQ infusion ports (Groeger et al, 19093). Moreover, when oral vs. intravenous treatment strategies have been assessed in other malignancies, patients strongly preferred oral agents as long as efficacy was not significantly compromised (Ishitobi *et al.*, 2013; Liu *et al.*, 1997). To this end, there has been significant interest in the development of an oral formulation of DNMTi. However, a major hurdle to establishing a stable oral bioavailability of these drugs is the rapid degradation and elimination by the enzyme cytidine deaminase (CDA), which is highly expressed in the gut and liver (Mahfouz *et al.*, 2013). Attempts to inhibit CDA with tetrahydrouridine (THU) have been attempted in sickle cell disease, before low-dose oral decitabine; however, THU has not been tested in malignancy and is unstable in acidic

environments (Molokie *et al.*, 2017). Another strategy to allow oral bioavailability has been attempted with AZA in an oral formulation (CC-486). The maximum tolerated dose in Phase 1 study in MDS was 480 mg/d, but mean relative oral azacitidine bioavailability was limited to 6.3%–20%, with 12.2% of patients experiencing grade 3/4 diarrhea deemed related to this high oral dose (Garcia-Manero *et al.*, 2011). Our further pursuits with CC-486 exhibited activity with lower, extended dosing, but with vastly divergent effects on methylation (than AZA) (Laille *et al.*, 2014; Savona *et al.*, 2018b). Thus, as co-administration of THU and DNMTi was not possible, and such high doses of CC-486 were necessary to overwhelm cytidine deaminase to approximate bioavailabilty of AZA, development of oral DNMTi had been stalled until a new oral CDA inhibitor (CDAi), cedazuridine, was synthesized with intention of administering in tandem with oral decitabine by Astex Pharmaceuticals. ASTX727 is an oral tablet fixed dose combination of decitabine (35mg) and cedazuridine (CDAi)(100mg). This innovation allows: 1) exploration of different dosing and home dosing schedules of DNMTi *outside* of the clinic for patient convenience, and 2) the opportunity to test all-oral combination therapies with any variety of the promising novel oral therapies using DNMTi therapy as a backbone in myeloid disease. We anticipate that this advance ultimately will lead to a reduction in health care costs, improved patient-reported outcomes and disease remissions, with lower toxicity than current standard of care.

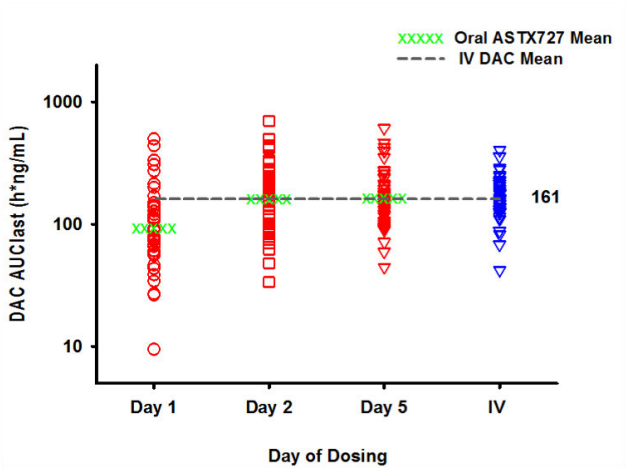


Figure 2: Mean AUC of ASTX727 vs DEC: Mean AUC calculated by pharmacokinetic analysis done on D1-D5 with ASTX727 and D1 of IV DEC.

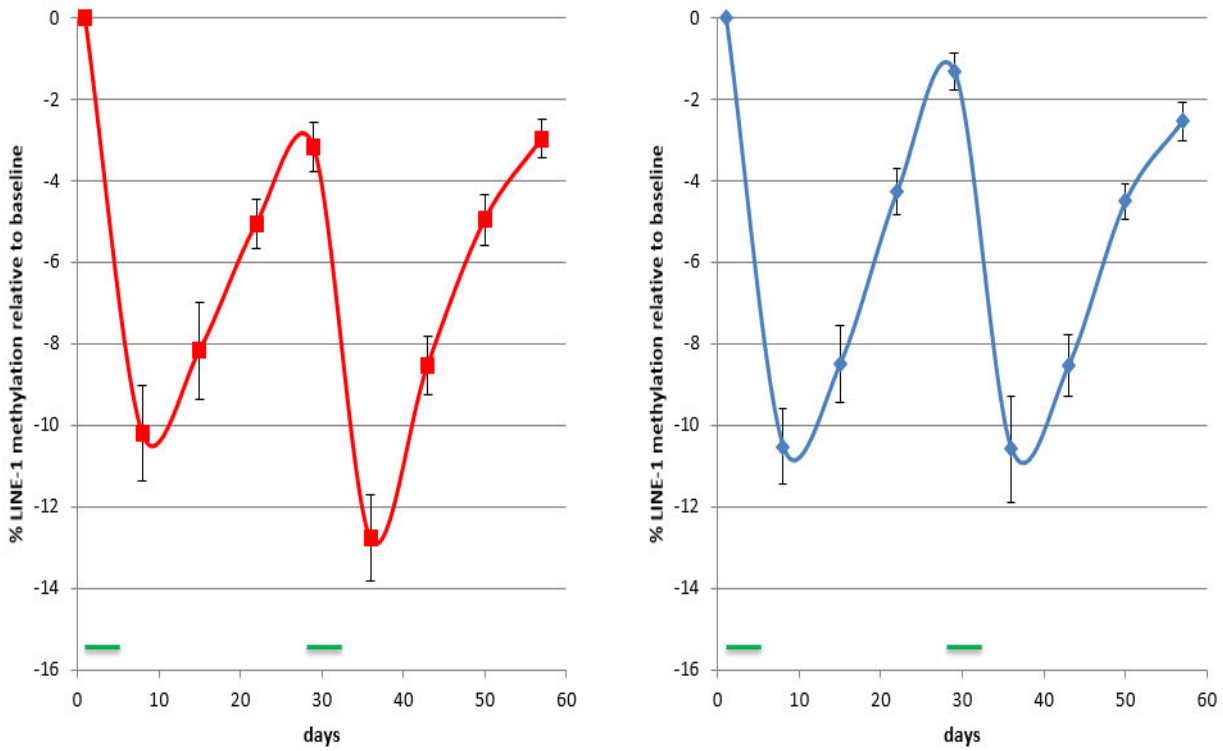


Figure 3: Preliminary Efficacy of ASTX727. Equivalent de-methylation by Line-1 Assay over time; DEC (left) and ASTX727 (right)

We have studied ASTX727 for the past 5 years through phase I-III trials in MDS. We conducted pharmacokinetics (pK)-driven studies to best understand bioavailability of ASTX727. In a phase I study, doses of decitabine and cedazuridine were titrated to approximate pharmacokinetic properties of parenteral decitabine (Savona *et al.*, 2019). The phase 2 dose of ASTX727 demonstrated bioavailability comparable to typical BSA-adjusted IV dosing, and preliminary evidence of efficacy with the oral DNMTi as expected with standard of care IV decitabine (Garcia-Manero *et al.*, 2020). The phase 2 and 3 design allowed patients the opportunity to receive oral DNMTi therapy for MDS after completing 2 cycles of pK-intensive monitoring in which patients were randomized to Group A (IV → PO) or Group B (PO → IV) to measure intra- and inter-patient variability in pK (**Figure 1**). In this experiment, DEC area under the curve (AUC) was equivalent between IV DEC and ASTX727 on day 2 – day 5 (D2-D5). AUC was found to approximate the AUC of IV DEC within 8 hours after treatment given the expected delays with oral therapy (**Figure 2**). The phase 2 experiment confirmed the decitabine systemic exposure equivalence between the oral ASTX727 fixed dose tablet and the IV decitabine BSA adjusted dose. The preliminary efficacy of ASTX727 from the phase 2 study was consistent with expected responses with DEC, and pharmacodynamic assessment via Line-1 de-methylation assay (Yang *et al.*, 2004) revealing similar patterns of de-methylation in the presence of ASTX727 and DEC (**Figure 3**) (Garcia-Manero *et al.*, 2020). Recently, the phase 3 study has confirmed the DEC systemic exposure equivalence (agreed with FDA as the primary endpoint) between ASTX727 and the IV decitabine BSA adjusted dose. The randomized intrapatient oral/IV AUC ratio was 98.9% - exceeding the regulatory required 90% CI equivalence range of 80-125% with a much narrower margin (90% CI of 92.7-105.6%) (Garcia-Manero *et al.*, 2019).

Nearly 200 patients have received ASTX727, and we continued to perform intricate pharmacokinetic studies to approximate the bioavailability of decitabine (DEC) (Garcia-Manero *et al*., 2020). Additionally, ASTX727 for treatment of AML is currently being explored in Europe. DEC has been successfully tested in combination with venetoclax in elderly patients with AML, and dosing has been established with a clear safety protocol (DiNardo *et al.,* 2019). These studies guided the construction of dosing levels and safety assessment of venetoclax+ASTX727 in younger patients.

2.4.3 Safety of Venetoclax administered with ASTX727

Neither decitabine nor cedazuridine inhibit CYP3A4 or P-gp, so no DDI is expected. Recently, new data generated by Astex internally addressed the potential effect of venetoclax on ASTX727 metabolism and again revealed no DDI. These data showed that venetoclax did not inhibit CDA (which is responsible for metabolism of decitabine), suggesting that venetoclax will not impact decitabine exposures when dosed concomitantly. Cedazuridine is not metabolized (only undergoes physical-chemical partial conversion to its epimer (non-enzymatic)), and neither cedazuridine, nor decitabine are substrates for CYPs. Thus, DDI is not expected in either direction.

A recent report from an ongoing Phase I study of the safety and preliminary efficacy of venetoclax + ASTX727 in older AML patients felt to be 'unfit' for induction chemotherapy (ClinicalTrials.gov Identifier: NCT04657081) has reported the safety profile of venetoclax + ASTX727 in older patients was similar to that seen in previous trials of venetoclax + parenteral azanucleosides previously explored in older AML patients (Mannis et al, ASH 2021). Co-administration of ASTX727 appeared to have no effect on steady state venetoclax AUC as the C2D5 (24,697 [84%] ng*hr/ml) and C2D15 (19,087 [61%] ng*hr/ml) AUC were comparable to published data (32,800 [52%] ng*hr/ml; Venclexta USPI). Although the steady state DEC AUC at C2D5 (277 [63%] ng*hr/ml) was slightly elevated compared to that observed in the Phase 3 Study (ASTX727-02), it was still within range of exposures seen on previous studies (189 [55%] ng*hr/ml; ASTX727-02 Clinical Study Report). Additionally, all AEs that were observed were expected in this patient population to arise from disease or from standard of care therapy of venetoclax+azanucleosides. Altogether, these data suggest there are no apparent drug interactions between venetoclax and ASTX727 that alter the expected drug exposures or AEs seen in previous venetoclax and DEC studies.

2.4.4 Leukemia Initiating Cells as the Source of Relapse

While some patients treated with venetoclax+DNMTi have long term disease free survival, it is clear that resistance emerges in most, and duration of response in these patients may often be short (DiNardo *et al.*, 2019; DiNardo *et al.*, 2018b; Wei *et al.*, 2019; Pollyea *et al.*, 2018). Thus, investigation of venetoclax -treatment failure remains a priority in AML research and care. Leukemia initiating cells (LICs) are important mediators of relapse in AML and MDS (Gentles *et al.*, 2010; Ng *et al.*, 2016), as they are not eliminated by conventional therapy (Chen *et al.*, 2018; Reyna *et al.*, 2017; Will *et al.*, 2012). We have shown that LICs have increased amounts

of anti-apoptotic proteins; thus providing additional rationale for the study of LICs in resistance to venetoclax +DNTMi (Shastri *et al.*, 2018). Mounting experimental data in various malignancies tells us that resistance to venetoclax often occurs in setting of upregulation of the BCL2 family antiapoptotic protein Induced Myeloid Cell Leukemia differentiation protein (MCL1), which is not targeted by (Horing *et al.*, 2017; Lin *et al.*, 2016; Konopleva *et al.*, 2016; Kirimura *et al.*, 2016; Jilg *et al.*, 2016). Moreover, our initial experience testing AML patient samples suggests that some patients will show de novo dependence on MCL1 or the related BCL2 family protein, BCL-XL. With the ongoing development of selective MCL1 inhibitors (Ramsey *et al.*, 2018; Caenepeel *et al.*, 2018; Kotschy *et al.*, 2016), identifying mechanisms of venetoclax resistance and understanding the dynamics of BCL2 homology

2.4.5 Experience with BCL2 inhibition in the clinic

The PI has tested venetoclax in early clinical development for the past 6-7 years starting with dose finding experiments in chronic lymphocytic leukemia, and our team at Vanderbilt led enrollment in the venetoclax +LDAC phase 1b trial in the United States (Wei *et al.*, 2019). Our team leads 2 investigator-sponsored clinical trial (IST) exploring the effects of non-specific MCL-1 inhibition or non-specific MCL-1 reduction combined and BCL2 inhibition: 1) a new study of venetoclax +selinexor across hematologic malignancies, 2) pevonedistat+AZA in MDS. Selinexor is a selective inhibitor of nuclear export which induces TP53, blocks translation of MCL-1 and BCL2 (via inhibition of transport of mRNA for both proteins from the nucleus), and has been shown to have efficacy across a wide variety of malignancies (Chen *et al.*, 2018; Garzon *et al.*, 2017; Kuruvilla *et al.*, 2017). We previously achieved CRR in 50% of newly diagnosed elderly AML patients with pevonedistat+AZA (Swords et al, 2018) and have an ongoing IST treating DNMTi-failure MDS with success with pevonedistat+AZA (NCT03238248). We also study the synergy of pevonedistat and venetoclax preclinically in collaboration with AbbVie scientists.

2.4.6 Dose Scheduling Rationale

2.4.6.1 ASTX727 Dosing

Based on clinical experience with ~200 patients, ASTX727 dosing will begin at 100 mg/35mg five times weekly. ASTX727 dosing in combination with other drugs is currently under evaluation in multiple studies, with doses and dosing schedule selected to optimize both tolerability and efficacy. Multiple combination studies have found that ASTX727 as little as three times weekly at 100mg/35mg dose in combination, can be given without loss of efficacy. Given overlapping toxicity with combination of both drugs, procedures similar to those followed in the VEN+DAC phase Ib study will be followed in this study.

2.4.6.2 Venetoclax Dosing

Venetoclax will be dosed according to standard dosing practices with one dose de-escalation option. Beginning on Cycle 1, Day 1 (after receiving mandatory anti-TLS hydration/prophylaxis), all patients are scheduled to initiate oral venetoclax on Days 1-5 of all Cycles. In Cycle 1 there will be a 3-day dose ramp-up: C1D1 100mg, C1D2 200mg, C1D3-21

OR C1D3-28 400mg. Subsequent cycles will be subject to continuous venetoclax dosing at 400mg/day.

2.5 Correlative Studies Background

2.5.1 Understanding response and resistance to venetoclax/DNMTi

Unfortunately, venetoclax failure is complex and, based on early experiments on patient samples, is not always hallmarked by clear upregulation of MCL1 (Ramsey *et al.*, 2018). Interestingly, our collaborators have shown that LICs from untreated patients are reliant on oxidative phosphorylation (OXPHOS) for survival (Lagadinou *et al.*, 2013). Many functionally defined LICs aberrantly overexpress BCL2, and it was shown that venetoclax's activity against BCL2 inhibits OXPHOS, thereby eradicating LICs. (Lagadinou *et al.*, 2013; Pollyea *et al.*, 2018). Specifically, venetoclax +AZA was shown to disrupt the TCA cycle and diminish oxygen consumption (Pollyea *et al.*, 2018; Stevens *et al.*, 2018). The resumption of OXPHOS has been shown to be a requirement in venetoclax+AZA-resistant cells (Farge *et al.*, 2017). While the increases in fatty acid oxidation (FAO) to salvage OXPHOS is seen in resistance (Stevens *et al.*, submitted; see C.2.a preliminary data), the contribution of MCL1 and role of LICs in this energy shift is not entirely clear. Additionally, while LICs overexpress BCL2, it is important to learn how other BCL2 family members, such as MCL1, BCL-XL, or BCL-A1, may influence maintenance of LICs or subclones. Our team showed that potent, selective MCL1 inhibitors may be effective against venetoclax -resistant AML, suggesting that cellular dependence on MCL1 may account for some of the resistance to venetoclax +DNMTi (Ramsey *et al.*, 2018; Teh *et al.*, 2018) Though we have shown that MCL1 promotes fatty acid metabolism (Escudero *et al.*, 2018) and subsequently regulates OXPHOS in other tumors (Lee *et al.*, 2017), the mechanism through which LICs regulate the switching of BCL2/MCL1 reliance in venetoclax-resistant AML, and its role in the regulatory control of LIC metabolism have not been thoroughly investigated. In our clinical correlate studies, we will test the hypothesis that phenotype-driven drug testing will reveal BCL2-resistant blasts within patient samples with MCL1 dependence; we will validate previous findings of a skewing toward FAO in venetoclax+AZA resistance within venetoclax +AST727 resistant samples; and we will discover LIC clones and subclones which portend resistant phenotypes and energy utilization in that resistance. In our final correlate study, we will conduct a co-clinical trial, leveraging over a decade of experience in generating AML PDX models, to simulate the effects of different therapy choices on untreated samples, when selection of therapy on the clinical trial was a failure (Ramsey *et al.*, 2018; Larochelle *et al.*, 2011).

2.5.2 Integrated Studies

2.5.2.1 DNA variants of Leukemic Initiating Cells (LIC).

The purpose of this study is to identify biomarkers of response/resistance by identifying mutations in primitive clonal cells pre and post treatment and correlate with response and transcriptomic signatures. The hypothesis is mutational burden within LICs will differ between responders and non-responders and may predict depth of response.
Particular genetic mutations commonly found in AML have been associated with VEN

response/resistance. Those harboring mutations in *TP53*, *RAS* family members, and *FLT3*-ITD tend to experience poorer response while those with *NPM1* or *IDH2* mutations often experience durable remissions (Dinardo *et al*, 2020; Chen *et al.*, 2019b; Nechiporuk *et al.*, 2019; Chyla *et al.*, 2018; Prata *et al.*, 2018; Daver *et al.*, 2013; and Nabinger *et al.*, 2019). Interestingly, ectopic expression of *FLT3*-ITD has also been shown to result in MCL1 dependence (Rowe *et al.*, 2010). Further, in our studies indicating that VEN treatment selects for monocytic disease, we also found *RAS* family mutants were only in the monocytic subclones in both pre and post VEN therapy (Lin *et al.*, 2016). Our sample size was small, and there is no indication from larger cohorts that *RAS* family mutations are exclusive to AML of monocytic lineage. However, given our metabolic data for these cell types, presence of these mutations may alter the biochemistry to affect response to BH3 mimetic therapy in monocytic cells. It is critical to identify the genomic determinants of response or resistance to treatment of AML with venetoclax +ASTX727 in younger patients at the level of LIC's.

These assays will be performed in the Verma/Steidl laboratory (not a CLIA laboratory) at Albert-Einstein Cancer Center. The Steidl and Verma laboratories have focused on LICs for many years and recently developed high resolution assays that can interrogate the transcriptional and mutational landscape of highly purified LICs. The use of flow sorting of aberrant LICs followed by deep DNA sequencing of 500 frequently mutated and functionally relevant genes in hematologic malignancies results in unprecedented sequencing depth at the stem cell level (equivalent to **~500,000x to 2,500,000x** depth at the bulk, unsorted AML) (Chen *et al*., 2019). We expect to obtain at least 36 patient samples with matched pre and post treatment (C1D8) with venetoclax +ASTX combination. Thus, as we project a CR/CRi of 67%, a conservative estimate of available samples is approximately 12 non-responders and 12 responders. We will use this approach to detect mutations in LICs present in samples taken from at least 10 venetoclax+ASTX727 responders and 10 non-responders pre- and post-treatment which will allow us to determine sub-clonal stem and progenitor cell types playing roles in resistance to the venetoclax +ASTX727 regimen. We will pair this with scRNAseq on the sorted LICs cells from these samples (Figure 4). scRNAseq will allow us to distinguish subtle transcriptome changes between different LIC subclones. We will compare samples from this post-treatment time point to diagnosis samples across both responders and non-responders to determine the metabolic-associated transcriptomic signatures that correlate with response or resistance. Additionally, we will overlay the transcriptomic data and somatic mutation data with patient response data to identify the transcriptome signatures and mutations corresponding with response or resistance to therapy. This will: 1) provide a non-biased approach to determine if changes in metabolic transcription programs occur at the level of the LIC with venetoclax + ASTX727/DEC

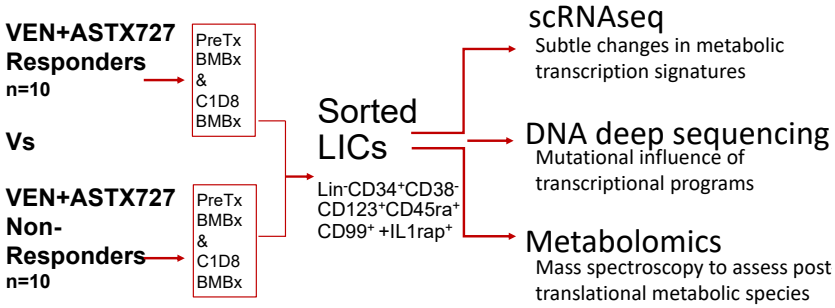


Figure 4: Experimental plan for biomarker sequencing studies. Cells from pre-treatment (PreTx) BMBx and C1D8 BMBx in responders and non-responders will be assessed. VEN=venetoclax.

as with venetoclax +AZA; 2) allow for identification of additional clinical or disease characteristics and somatic mutations potentially associated with specific metabolic dependencies which may subsequently be used as biomarkers; 3) characterize the subclonal mutational changes in stem and progenitor cells that will correlate with response and resistance. While our preliminary data point to cellular metabolism, we will also interrogate the data for undiscovered gene expression alterations. These data will be further considered for analyses with the results of *ex vivo* drug response data and PDX responses to the alternative treatment arm.

We propose to assess LICs from BMMC specimen by scRNAseq and deep DNAseq from patient samples collected at baseline/screening and at cycle 1 day 8.

2.5.3 Exploratory Studies

2.5.3.1 LIC transcriptomic signatures

This study aims to identify biomarkers of response/resistance through transcriptomic signatures in responders vs non-responders. The hypothesis of these studies is LICs in responders vs non-responders to venetoclax+ASTX727 will have different cell maturation, metabolic and cell cycle transcriptomic signatures as determined by scRNAseq.

AML is frequently incurable and is characterized by a high rate of refractory and relapsed disease after conventional therapies. Recent experimental evidence including work from within our own group has shown that disease initiating stem cells/LICs in AML can exist as pools of relatively quiescent cells that do not respond well to common cell-toxic agents and thereby contribute to treatment failure (Will *et al.*, 2012; Pang *et al.*, 2013; Jan *et al.*, 2012; Woll *et al.*, 2014; Shlush *et al.*, 2014; Elias *et al.*, 2014; Craddock *et al.*, 2013). As a consequence, understanding the molecular makeup of aberrant stem and progenitor cells is important to achieve a lasting cure for these myeloid disorders. Many factors have been suggested to increase risk of resistance to venetoclax+DNMTi including RAS family activation, loss of functional TP53, mitochondrial structural changes, and upregulation of MCL1 (Nechiporuk *et al.*, 2019; Chen *et al.*, 2019). We postulate that these mutations and structural adaptations ultimately lend the ability to alternate energy use in the face BCL2 inhibition. We have previously shown that MCL1 plays a role in fatty acid metabolism in other tumors (Escudero *et al.*, 2018) and in our collaboration with the Jordan Lab, established that FAO is an independent risk factor for AML resistance to venetoclax+AZA. *Responsive* AML LICs have insufficient capacity to upregulate fatty acid oxidation (FAO), whereas *resistant* cells resume cellular respiration with fuel from fatty acids, thus negating the biochemical consequences of venetoclax+AZA therapy against AML LICs (Pei *et al.*, 2020). Selective inhibition of MCL1 with VU661013 shuts down FAO and reduces relative oxygen consumption (OCR) in venetoclax-resistant AML (**Figure 5**).

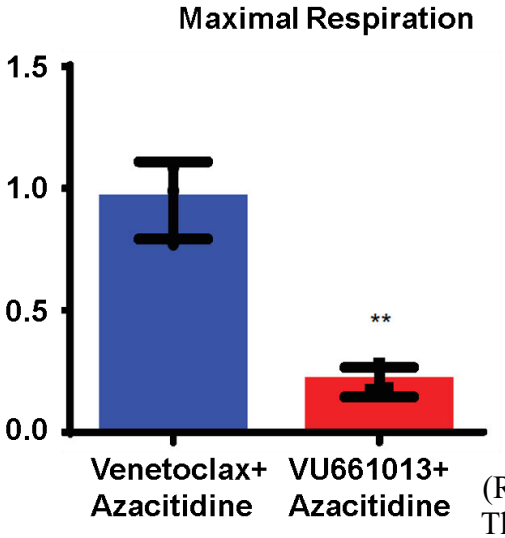


Figure 5: Venetoclax-reistant AML: Venetoclax+AZA does not reduce O_2 consumption whereas VU661013+AZA significantly diminishes OCR.

It is known that MCL1 dependency increases as cells mature in normal hematopoiesis (Lara-Astiaso *et al.*, 2014; Novershtern *et al.*, 2011). Previously, we observed distinct differences in BCL2 and MCL1 expression between AML subtypes where less mature subtypes (AML001) express high levels of BCL2 and very little MCL1, and mature monocytic subtypes (AML002) express very little BCL2 and high levels of MCL1 (**Figure 6**) (Ramsey *et al.*, 2018). This was recently confirmed by another group using analysis of samples in TCGA. They further showed that *ex vivo* response to venetoclax also correlated with less mature AML subtypes in the BEAT AML dataset (Kuusanmaki *et al.*, 2019). Moreover, we found that *in vivo* response to venetoclax inhibition in PD X models also corresponds with subtype (**Figure 7**). Taken together these data highlight the importance of evaluating specific cell populations within a sample.

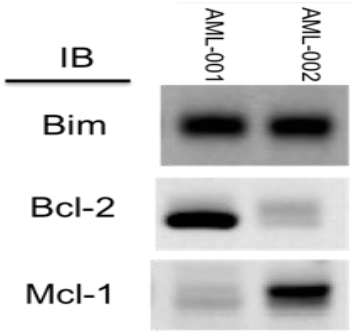


Figure 6. BCL2, MCL1 proteins differentially expressed in different subtypes of AML

Though we have shown that MCL1 plays a role in fatty acid metabolism(Escudero *et al.*, 2018) and does regulate OXPHOS in other tumors (Lee *et al.*, 2017) the dynamic regulation of LIC metabolism and use of key metabolic pathways that may promote cell survival and therapy resistance, particularly in venetoclax+decitabine/ASTX727 treatment, remains poorly understood. We have established expertise in metabolism and mitochondrial function to explore this (Escudero *et al.*, 2018; Adams *et al.*, 2016; Deming *et al.*, 2006; Gerriets et al, 2016; Johnson *et al.*, 2018; Kishton *et al.*, 2016; Lindsten *et al.*, 2000; Rathmell *et al.*, 2000; Schulte *et al.*, 2018; Siska, Beckermann *et al.*, 2017; Siska, van der Windt *et al.*, 2016.

Importantly, it is not known whether specific gene transcription programs or somatic mutations in LICs can predict OXPHOS-dependent cell death and subsequently act as potential biomarkers of response. It is critical to identify the genomic determinants of response or resistance to treatment of AML with venetoclax +ASTX727. We must evaluate the potential relationship between the efficacy of venetoclax+ASTX727 and gene expression dynamics in LICs as it has been shown that venetoclax+AZA targets LICs by limiting their capacity to support OXPHOS (Ramsey *et al.*, 2018; Stevens *et al.*, 2018). To understand how metabolism may associate with potential resistance to venetoclax+ASTX727 it is necessary to analyze LIC at a single cell level. We propose to identify AML-specific somatic alterations and transcriptomic changes in responders and non-responders to venetoclax+ASTX727 with deep DNA sequencing and single-cell RNA sequencing. *Our preliminary data suggests a connection between a role of MCL1-mediated FAO in venetoclax resistance, and scRNAseq will allow us to simultaneously evaluate the expression of relevant genes in cells of interest in these leukemia initiating cells.* Our transcriptome analysis will be conducted in an unbiased fashion, but we will focus on genes involved in cellular metabolism in attempts to define a biomarker. We will analyze these data in concert with the proposed *ex vivo* drug response and *in vivo* PDX response. As many energy determinants, such as metabolites, cannot be properly assessed by transcriptome analysis, we will further validate our transcriptomic findings with targeted mass spectrometry. By applying these paired methods to pre- and post-treatment patient samples, we aim to identify clonal aberrations that may serve as a biomarker of response and further understand the regulatory networks behind metabolic plasticity in LICs, mutational influence of energy use, and how this may lead to drug resistance.

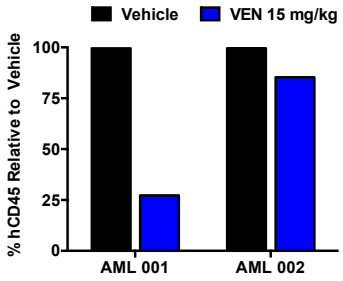


Figure 7: Venetoclax (VEN) effectively inhibits less mature subtypes of AML *in vivo* (AML001) but is ineffective against monocytic AML (AML002)

scRNAseq of LICs will allow us to distinguish subtle transcriptome changes between different LIC subclones. We will compare samples from this post-treatment time point to diagnosis samples across both responders and non-responders to determine the metabolic-associated transcriptomic signatures that correlate with response or resistance. Additionally, we will overlay the transcriptomic data and somatic mutation data with patient response data to identify the transcriptome signatures and mutations corresponding with response or resistance to therapy. This will: 1) provide a non-biased approach to determine if changes in metabolic transcription programs occur at the level of the LIC with venetoclax+ASTX727/DEC as with venetoclaz+AZA; 2) allow for identification of additional clinical or disease characteristics and somatic mutations potentially associated with specific metabolic dependencies which may subsequently be used as biomarkers; 3) characterize the subclonal mutational changes in stem and progenitor cells that will correlate with response and resistance. While our preliminary data point to cellular metabolism, we will also interrogate the data for undiscovered gene expression alterations.

2.5.3.2 Morphology/maturation and drug sensitivity.

The aim of this study is to identify biomarkers of response via a high-throughput, immunophenotypic assessment of drug sensitivity, revealing response of each cell type and stage

of disease maturation. The hypothesis being tested by this study is flow cytometry-based ex vivo drug testing can predict patient response to VEN+ASTX727, MCL1 inhibition, or 7+3. This approach allows us to assess the response of the leukemic cell populations specifically as well as the response of leukemic cells in differing stages of maturation.

<u>High throughput immunophenotyping drug response assessment</u>

Experimental data in various malignancies suggests that venetoclax resistance most commonly occurs in the presence of increased expression of *MCL1*, which is not targeted by venetoclax (Horing *et al.*, 2017; Lin *et al.*, 2016; Konopleva *et al.*, 2016; Kirimura *et al.*, 2016; Jilg *et al.*, 2016). Given early adoption of venetoclax on trial, and treatment of 50 AML patients off study with venetoclax+DNMTi based regimens, we collected ample patient samples to begin to study of the role of MCL1 in resistance to venetoclax. This was enabled by a partnership with chemist Stephen Fesik, a pioneer in BCL2 family drug discovery and Professor at Vanderbilt University, who developed the first pan-BCL2 inhibitors (Chang *et al.*, 1997; Fesik *et al.*, 2005; Oltersdorf et al, 2005; Petros et al, 2001) and, most recently, a novel MCL1 inhibitor series (Ramsey *et al.*, 2018; Lee *et al.*, 2017; Shaw *et al.*, 2018; Zhao *et al.*, 2018). In collaboration with the Fesik laboratory, we led translational studies to exhibit the outgrowth of MCL1 dependent AML cells after selective pressure from venetoclax, and, conversely, BCL2 dependence in cells treated

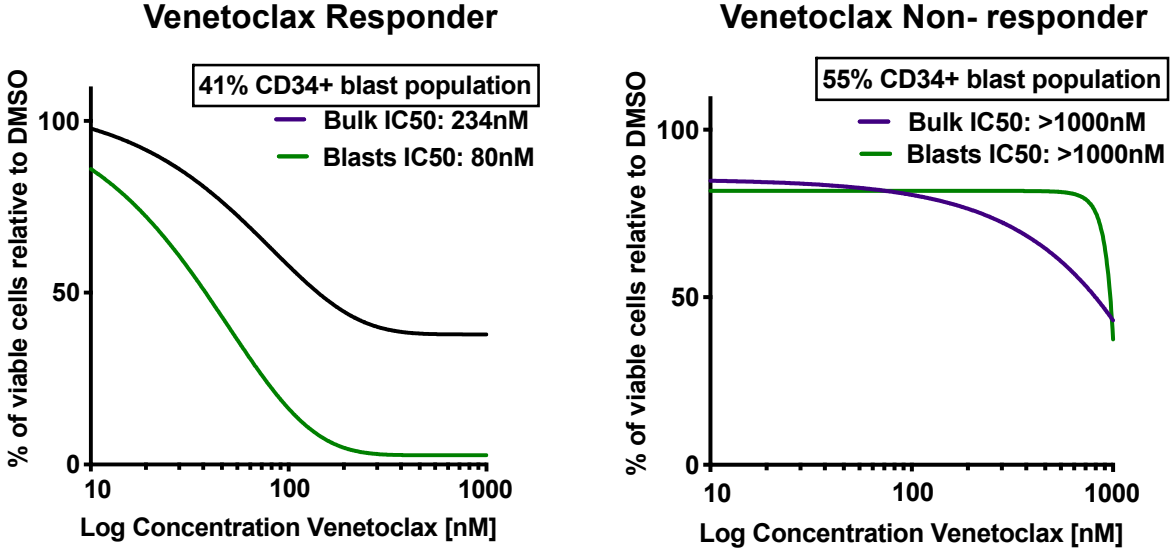


Figure 8: Response to venetoclax within bulk and blast AML populations in venetoclax-resistant and venetoclax-sensitive patients. Percentage of blasts in the sample population is stated.

with MCL1 inhibitor. We conducted preliminary experiments with combinations of both agents and continue to study the safety and efficacy of that combination preclinically (Ramsey *et al.*, 2018; Lee *et al.*, 2017). In addition, given the advent of these potent, selective inhibitors of BCL2 and MCL1, we have generated dose response curves for various leukemia cell lines and AML patient samples treated with each selective inhibitor using CellTiter-Glo®. However, in testing these patient samples *in vivo*, we observed frequent discordance between *in vitro* and *in vivo* results. As AML is an extremely heterogeneous disease, neither bulk viability nor apoptosis assays provide the data granularity needed to reproducibly predict drug efficacy in patient samples. To improve upon this, we employed a cell phenotype-based flow cytometry method to

determine the efficacy of venetoclax on multiple cell populations simultaneously within a given sample in a high-throughput fashion. Preliminary experiments on venetoclax-sensitive and venetoclax-resistant patients indicate that this phenotype-driven analysis should allow for specific assessment of relevant blast populations to more accurately predict patient response to venetoclax (**Figure 8**).

Methods assessing response of specific AML cell populations may more accurately reflect in vivo response (Kuusanmaki *et al.*, 2019). Here, we propose to use the Intellicyt® iQue Screener PLUS to evaluate drug efficacy on specific subpopulations within individual patient samples with ≤3uL of sample, allowing for efficient use of precious resources, and longitudinal assessments. Based on our preliminary data (Fig. 5-8), we hypothesize that by more precisely determining the specific cell populations responding to the drug, we will more accurately identify or predict responders in the clinical trial. If response in this assay correlates with the patient response, we will further evaluate the drug response of individual subpopulations within each AML alongside transcriptome and metabolic analyses which will strengthen our determination of biomarkers.

Flow-based drug screen:
This assay will be performed in the Savona laboratory (not a CLIA laboratory) at Vanderbilt University Medical Center. The Savona laboratory has extensive experience in handling and testing drug sensitivity of AML patient samples by flow cytometry. With this new approach, we will determine the efficacy of Venetoclax+ASTX727, '7+3', and other selective BCL2 family inhibitors on multiple cell populations (blasts, monocytes, etc.) simultaneously within an AML patient pre-treatment sample. We will seed cryopreserved AML Bone marrow mononuclear cells (BMMCs) into 96 well plates in mononuclear cell medium supplemented with 10μg/mL gentamicin and 2.5 μg/mL amphotericin B and treat cells with a dynamic range of increasing concentrations of each drug via an Echo® 550 acoustic liquid handler. We will treat with a monotherapy (Venetoclax, ASTX727, cytarabine, daunorubicin, MCL1 inhibitor, etc), and then combination therapy, as appropriate. Pre-treatment, after 48hrs and again at 72hrs of treatment, we will stain the cells with the following cell surface markers: CD33, CD45, CD14, CD38, and CD34 to specifically identify blasts, monocytes, and immature granulocytes followed by Annexin V. Flow cytometry analysis will then be conducted using the Intellicyt® iQue instrument. ForeCyt® software is used for gating and cell counts. The cell count of each well will be normalized to the average of all adjacent DMSO controls.

These experiments will be conducted on cryopreserved samples from an estimated maximum 80 of the 96 available pre-treatment samples, (based on 95% recovery; accounting for assumed frequency of sample availability, and failure of test rate – based on previous translational studies in AML conducted at our institution).

2.5.3.3 Alternative therapy response

The aim of this study is to determine if treatment failure is a function of therapy choice and if treatment failure results in resistance to the alternative therapy. The hypothesis for this study is

PDX models can predict patient response and provide preliminary data regarding the sequencing of chemotherapy and VEN+ASTX727 for future trials. These PDX experiments will allow a direct comparison of response to VEN+ASTX727 in the treatment naïve and chemo relapse/refractory setting.

PDX resource and experience

Patient derived xenografts (PDX) in immunocompromised murine models provide instrumental avatars for patient response to therapy and will be leveraged to study samples from resistant patients from this clinical trial. Here we propose the use of a co-clinical trial with PDX models generated with cells taken from clinical trial patients who ultimately did not respond to their assigned intervention. The use of PDX co-clinical trials to aid in clinical trial design and inform on patient outcome is still a relatively new approach but one that has been validated to replicate the patient's disease (Scott *et al.*, 2013; Weroha *et al.*, 2014; Glaser *et al.*, 2015; Hidalgo *et al.*, 2014; Rego, Warrell, *et al.*, 2000; Rego, He, *et al.*, 2000; Hidalgo *et al.*, 2011). The study team has extensive experience with immunocompromised mouse models, (Ramsey *et al.*, 2018; Reyna *et al.*, 2017; Chen *et al.*, 2019; Antony-Debre *et al.*, 2017; Carter, McNamara *et al.*, 2011; Carter *et al.*, 2010; Will *et al.*, 2015) and designing PDX experiments to test efficacy of pharmaceutical agents (Ramsey *et al.*, 2018; Chen *et al.*, 2019; Fischer *et al.* 2020; Ramsey *et al.*, 2020; Ramsey et al. (in press); Ramsey *et al.*, submitted; Shenoy *et al.*, 2019; Carvajal *et al.*, 2018). The Savona laboratory has completely characterized a library of more than 25 genetically diverse P0 PDXs for transplantation and experimentation, and our team has conducted over 3000 intravascular, intraperitoneal or intrafemoral transplants into NSG or NSGS (NSG-SGM3, NOD-scid IL2Rgnull-3/GM/SF) mice which are genetically engineered to express human cytokines (GM-CSF, SCF, IL3) on a NOD-Scid-IL2 gamma receptor knockout background for superior engraftment of human hematopoietic malignancies (Wunderlich *et al.*, 2010). Overall, the Savona Laboratory is well prepared to conduct the proposed experiments.

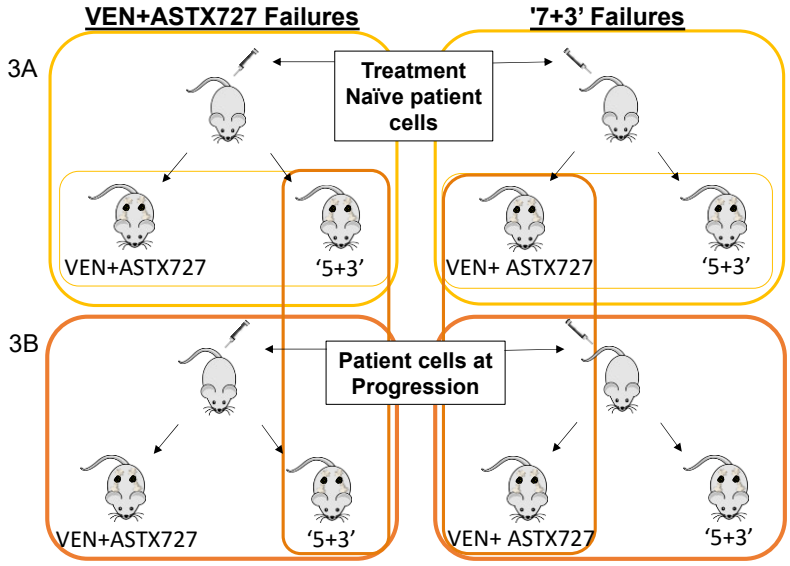


Figure 9: PDX Co-clinical trial design. Patients with treatment failure will be assessed by PDX co-clinical trials. We will engraft mice with cells collected pre-treatment, treat them with both interventions, and assess response (yellow boxes). For the cross-over trial simulation, we will engraft mice with cells collected from the patient at the time of progression, treat them with both interventions, and assess response between the alternate intervention in the treatment naïve setting vs the treatment refractory setting (orange boxes).

Clinical trials testing venetoclax-based regiments in AML have consistently reported significantly reduced response rates in relapsed/refractory patients compared to treatment naïve

patients, (DiNardo *et al.*, 2019, Wei *et al.*, 2019; Pollyea *et al.*, 2018; Ram *et al.*, 2019; Mei *et al.*, 2019; DiNardo *et al.*, 2018c) but the reasons for this are unclear. Likewise, as patients treated with venetoclax+DNMTi on clinical trial were deemed not fit for cytotoxic chemotherapy induction, very few later received cytotoxic chemotherapy; thus, the capacity to salvage venetoclax+DNMTi failure with '7+3' is unknown. Therapy sequence decisions are a critical piece in optimizing a patient's treatment plan, and determining the full implications of a prior therapy toward response of a subsequent therapy is critical to making advances in the refinement of therapeutic decisions. Thoughtful use of PDX trials to can help answer questions that cannot be addressed in the clinic. Here we have designed a PDX co-trial simulation to provide data to inform these decisions (**Figure 9**). In our 'co-clinical trial' with PDXs we can parse patient samples among competing murine cohorts and simulate the testing of both trial therapies in one patient.

PDX transplantation: Female NSGS [NOD-scid IL2Rgnull3Tg (hSCF/hGM-CSF/hIL3)] mice, 6-8 weeks old will be irradiated with 100 cGy radiation. Twenty-four hours later, mice will be transplanted via tail vein injection with approximately 2 million mononuclear AML cells. Mice will be randomized post cell injection into cages of 5 and monitored weekly for establishment of chimerism.

*PDX Co-Clinical Trial design (**Figure 9**)*: All biospecimens used to generate PDX models will come from patients who do not respond to their assigned therapy arm. We will generate competing PDX models using cells taken prior to therapy and at treatment failure. Models generated from pre-treatment and treatment failure cells will be divided into 7 treatment groups and receive the following treatments based on MTD for NSGS models: vehicle, VEN, ASTX727, VEN+ASTX727, cytarabine, daunorubicine, or cytarabine+daunorubicin. We will follow the previously described dosing schedule, as tolerated by mice, of daunorubicin for 3 days and cytarabine for 5 days (in place of '7+3') (Wunderlich et al., 2013; Toldo et al., 2013). There is not a standard method for applying hematologic response assessments in the clinic to a PDX. While we will know prior to engraftment that a patient did not respond to their assigned intervention, we will still include a PDX treatment group with the patient's assigned intervention as a proper control measure of response for the model in both the co-clinical trial and the cross-over simulation. Mice will be monitored weekly for peripheral chimerism, weight and body condition scoring. As not all AML PDX generate peripheral chimerism despite clear bone marrow chimerism, we will perform serial bone marrow aspirates to monitor response. We will allow mice to continue on treatment until morbidity limits defined by the IACUC and assess the experiment using Kaplan Meier analysis.

The known engraftment failure rate in NSGS mice for AML PDX is 50%. This limits the number of PDXs which can be generated from treatment failures from the clinical trial. Despite this, we estimate that we can successfully generate 10 PDX models from the VEN+ASTX727 failed patients and 10 PDX models from the '7+3' failed patients.

2.5.4 Venetoclax Pharmacokinetic Studies

Venetoclax has low bioavailability, high inter-indiviaul pharmacokinetic variability, and is susceptibility to drug-drug interactions through inhibition of intestinal/liver CYP3A4, P-

glycoprotein, and BCRP and liver CYP3A4 and OATP1B1. The unpredictable PK properties of venetoclax, primarily through high DDI potential warrant detailed plasma pharmacokinetic evaluation. This will allow for the assessment of exposure-toxicity/efficacy associations during AML therapy.

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Subjects must be between 18-65 years of age at the time of signing the Informed Consent Form (ICF) and must be able to meet all study requirements. AML patients under the age of 18 are excluded as is being studied in in patients under 18 years of age in different venues.

3.1.2 Morphologically confirmed diagnosis of AML in accordance with WHO diagnostic criteria.

3.1.3 Adverse risk AML per 2017 ELN Recommendations (Appendix A).

3.1.4 Subjects must be either treatment naïve defined by ≤ 1 cycle of DNMTi therapy, no history of cytotoxic chemotherapy for their AML; prior treatment with lenalidomide, hydroxyurea or ESAs is allowed (prior treatment for MDS with >1 cycle of DNMTi is not allowed).

3.1.5 A bone marrow aspirate and biopsy must be performed, and tissue collected for entrance to the trial.

3.1.6 ECOG performance status ≤2 (Karnofsky ≥60%, see Appendix B).

3.1.7 Recovery to ≤ Grade 1 or baseline of any toxicities due to prior systemic treatments, excluding alopecia.

3.1.8 Patients must have adequate organ and marrow function as defined below:

- WBC <25,000 (may be reduced with leukopheresis or hydroxyurea prior to study start)
- direct bilirubin ≤ 2 × institutional upper limit of normal (ULN)
- AST(SGOT)/ALT(SGPT) ≤3 × institutional ULN
- Creatinine clearance ≥30 mL/min (per the Cockcroft-Gault formula – Appendix C)

3.1.9 Human immunodeficiency virus (HIV)-infected patients on effective anti-retroviral therapy with undetectable viral load within 6 months are eligible for this trial.

3.1.10 For patients with evidence of chronic hepatitis B virus (HBV) infection, the HBV viral load must be undetectable on suppressive therapy, if indicated.

3.1.11 Patients with a history of hepatitis C virus (HCV) infection must have been treated and cured. For patients with HCV infection who are currently on treatment or have received treatment, they are eligible if they have an undetectable HCV viral load.

3.1.12 Tumor lysis present prior to therapy must be treated accordingly prior to start of therapy

3.1.13 The effects of venetoclax and ASTX727 on the developing human fetus are unknown. For this reason and because BCL2 inhibitor and DNMTi agents as well as other therapeutic agents used in this trial (cytarabine and daunorubicin) are known to be teratogenic, women of child-bearing potential must agree to use adequate contraception (hormonal birth control or abstinence) prior to study entry and for the duration of study participation, and for 6 months following completion of study treatment. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men treated or enrolled on this protocol must also agree to use adequate contraception (latex or synthetic condom or abstinence) prior to the study, for the duration of study participation, and 3 months after completion of venetoclax and ASTX727 administration.

3.1.14 Ability to understand and the willingness to sign a written informed consent document.

3.1.15 Adequate cardiac systolic function as measured by ejection fraction (EF) ≥50%.

3.2 Exclusion Criteria

3.2.1 Favorable or intermediate risk AML as defined by 2017 ELN criteria.

3.2.2 Presence of FLT3 TKD or FLT-ITD mutations.

3.2.3 Inability to tolerate oral medication or keep a pill diary.

3.2.4 Active documented central nervous system (CNS) leukemia.

3.2.5 Concurrent treatment with a non-permitted concomitant medication.

3.2.6 Concurrent anticancer treatment, major surgery, or use of any investigational drug within 28 days before the start of trial treatment.

3.2.7 Other malignancy currently being treated or likely to be treated in next 6 months with the exception of basal or squamous cell carcinoma of the skin or cervical carcinoma in situ and patients receiving hormonal therapy for prevention of hormone-sensitive cancers.

3.2.8 History of allergic reactions attributed to compounds of similar chemical or biologic composition to venetoclax, ASTX727, or other agents used in study.

3.2.9 Patient must not have received known moderate or strong CYP3A inducers within 7 days of enrollment. Patient must not have known medical conditions requiring chronic therapy of moderate CYP3A inducers. As part of the enrollment/informed consent procedures, the patient will be counseled on the risk of interactions with other agents, and what to do if new medications need to be prescribed or if the patient is considering a new over-the-counter medicine or herbal product.

3.2.10 Patients with uncontrolled intercurrent illness.

3.2.11 Patients with psychiatric illness/social situations (including substance abuse) that would limit compliance with study requirements.

3.2.12 Pregnant women are excluded from this study because venetoclax and ASTX727 have the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with venetoclax, breastfeeding should be discontinued if the mother is treated with venetoclax. These potential risks may also apply to other agents used in this study.

3.2.13 Previous exposure to either venetoclax or >1 cycle of DNMTi (*e.g.* azacitidine, decitabine, ASTX727, CC486).

3.2.14 Active, uncontrolled infection as determined by the investigator. Patients with infection under active treatment and controlled with antibiotics are eligible.

3.2.15 Any condition deemed by the investigator to make the patient a poor candidate for clinical trial and/or treatment with investigational agents.

3.3 Inclusion of Women and Minorities

NIH policy requires that women and members of minority groups and their subpopulations be included in all NIH-supported biomedical and behavioral research projects involving NIH-defined clinical research unless a clear and compelling rationale and justification establishes to the satisfaction of the funding Institute & Center (IC) Director that inclusion is inappropriate with respect to the health of the subjects or the purpose of the research. Exclusion under other circumstances must be designated by the Director, NIH, upon the recommendation of an IC Director based on a compelling rationale and justification. Cost is not an acceptable reason for exclusion except when the study would duplicate data from other sources. Women of childbearing potential should not be routinely excluded from participation in clinical research. Please see http://grants.nih.gov/grants/funding/phs398/phs398.pdf.

4. REGISTRATION PROCEDURES

4.1 Investigator and Research Associate Registration with CTEP

Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all individuals contributing to NCI-sponsored trials to register and to renew their registration annually. To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account at https://ctepcore.nci.nih.gov/iam. In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) at https://ctepcore.nci.nih.gov/rcr.

RCR utilizes five person registration types.

- IVR: MD, DO, or international equivalent,
- NPIVR: advanced practice providers (*e.g.*, NP or PA) or graduate level researchers (*e.g.*, PhD),
- AP: clinical site staff (*e.g.*, RN or CRA) with data entry access to CTSU applications such as the Roster Update Management System (RUMS), OPEN, Rave, acting as a primary site contact, or with consenting privileges,
- Associate (A): other clinical site staff involved in the conduct of NCI-sponsored trials, and
- Associate Basic (AB): individuals (*e.g.*, pharmaceutical company employees) with limited access to NCI-supported systems.

RCR requires the following registration documents:

Documentation Required	IVR	NPIVR	AP	A	AB
FDA Form 1572	✓	✓			
Financial Disclosure Form	✓	✓	✓		
NCI Biosketch (education, training, employment, license, and certification)	✓	✓	✓		
GCP training	✓	✓	✓		
Agent Shipment Form (if applicable)	✓				
CV (optional)	✓	✓	✓		

An active CTEP-IAM user account and appropriate RCR registration is required to access all CTEP and Cancer Trials Support Unit (CTSU) websites and applications. In addition, IVRs and NPIVRs must list all clinical practice sites and Institutional Review Boards (IRBs) covering their practice sites on the FDA Form 1572 in RCR to allow the following:

- Addition to a site roster,

- Assign the treating, credit, consenting, or drug shipment (IVR only) tasks in OPEN,
- Act as the site-protocol Principal Investigator (PI) on the IRB approval, and
- Assign the Clinical Investigator (CI) role on the Delegation of Tasks Log (DTL).

In addition, all investigators acting as the Site-Protocol PI (Investigator listed on the IRB approval), consenting/treating/drug shipment investigator in OPEN, or as the Clinical Investigator (CI) on the DTL must be rostered at the enrolling site with a participating organization.

Additional information is located on the CTEP website at https://ctep.cancer.gov/investigatorResources/default.htm. For questions, please contact the **RCR Help Desk** by email at RCRHelpDesk@nih.gov.

4.2 Site Registration

This study is supported by the NCI Cancer Trials Support Unit (CTSU).

IRB Approval

Sites participating with the NCI Central Institutional Review Board (NCI CIRB) must submit the Study Specific Worksheet (SSW) for Local Context to the CIRB using IRBManager to indicate their intent to open the study locally. The NCI CIRB's approval of the SSW is automatically communicated to the CTSU Regulatory Office, but sites are required to contact the CTSU Regulatory Office at CTSURegPref@ctsu.coccg.org to establish site preferences for applying NCI CIRB approvals across their Signatory Network. Site preferences can be set at the network or protocol level. Questions about establishing site preferences can be addressed to the CTSU Regulatory Office by email or calling 1-888-651-CTSU (2878).

In addition, the Site-Protocol PI (*i.e.*, the investigator on the IRB/REB approval) must meet the following criteria to complete processing of the IRB/REB approval record to be completed:

- Holds an active CTEP status,
- Active status at the site(s) on the IRB/REB approval (*applies to US and Canadian sites only*) and on at least one participating organization's roster,
- If using NCI CIRB, active on the NCI CIRB roster under the applicable CIRB Signatory Institution(s) record,
- Includes the IRB number of the IRB providing approval in the Form FDA 1572 in the RCR profile,
- Lists all sites on the IRB/REB approval as Practice Sites in the Form FDA 1572 in the RCR profile; and
- Holds the appropriate CTEP registration type for the protocol.

Additional Requirements

Additional site requirements to obtain an approved site registration status include:

- An active Federalwide Assurance (FWA) number,

- An active roster affiliation with the Lead Protocol Organization (LPO) or a Participating Organization (PO),
- An active roster affiliation with the NCI CIRB roster under at least one CIRB Signatory Institution (US sites only); and
- Compliance with all protocol-specific requirements (PSRs).

4.2.1 Downloading Site Registration Documents

Download the site registration forms from the protocol-specific page located on the CTSU members' website. Permission to view and download this protocol and its supporting documents is restricted to institutions and its associated investigators and staff on a participating roster. To view/download site registration forms:

- Log in to the CTSU members' website (https://www.ctsu.org) using your CTEP-IAM username and password,
- Click on *Protocols* in the upper left of the screen
  - Enter the protocol number in the search field at the top of the protocol tree, or
  - Click on the By Lead Organization folder to expand, then select Yale University Cancer Center and protocol number 10417.
- Click on *Documents*, Protocol Related Documents, and use the Document Type filter and select *Site Registration*, to download and complete the forms provided. (Note: For sites under the CIRB, IRB data will load automatically to the CTSU.)

4.2.2 Protocol Specific Requirements For Protocol 10417 Site Registration

- Specimen Tracking System Training Requirement:
  - All data entry users (Clinical Research Associate role) at each participating site will need to complete the Theradex-led training.
  - Theradex will provide a certificate of completion, which will need to be submitted to the CTSU through the Regulatory Submission Portal.
  - The training is a one-time only requirement per individual. If an individual has previously completed the training for another ETCTN study, the training does not need to be completed again nor does the certificate of completion need to be resubmitted to the CTSU. However, new versions of the Specimen Tracking System may require new training.
  - This training will need to be completed before the first patient enrollment at a given site.
  - Please contact STS Support at Theradex for the training (STS.Support@theradex.com, Theradex phone: 609-799-7580).

4.2.3 Submitting Regulatory Documents

Submit required forms and documents to the CTSU Regulatory Office using the Regulatory Submission Portal on the CTSU members' website.

To access the Regulatory Submission Portal, log on to the CTSU members' website, go to the

Regulatory section, and select Regulatory Submission.

Institutions with patients waiting that are unable to use the Regulatory Submission Portal should alert the CTSU Regulatory Office immediately by phone or email: 1-866-651-CTSU (2878), or CTSURegHelp@coccg.org

Delegation of Tasks Log (DTL)

Each site must complete a protocol-specific DTL using the DTL application in the Delegation Log section on the CTSU members' website. The Clinical Investigator (CI) is required to review and electronically sign the DTL prior to the site receiving an approved site registration status and enrolling patients to the study. To maintain an approved site registration status the CI must re-sign the DTL at least annually and when a new version of the DTL is released; and activate new task assignments requiring CI sign-off. Any individual at the enrolling site on a participating roster may initiate the site DTL. Once the DTL is submitted for CI approval, only the designated DTL Administrators or the CI may update the DTL. Instructions on completing the DTL are available in the Help Topics button in the DTL application and include a Master Task List, which describes DTL task assignments, CI signature, and CTEP registration requirements.

4.2.4 Checking Site Registration Status

Site's registration status may be verified on the CTSU website.

- Click on *Regulatory* at the top of the screen,
- Click on *Site Registration*, and
- Enter the site's 5-character CTEP Institution Code and click on Go.
    - Additional filters are available to sort by Protocol, Registration Status, Protocol Status, and/or IRB Type.

Note: The status shown only reflects institutional compliance with site registration requirements as outlined within the protocol. It does not reflect compliance with protocol requirements for individuals participating on the protocol or the enrolling investigator's status with NCI or their affiliated networks.

4.3 Patient Registration

4.3.1 OPEN / IWRS

The Oncology Patient Enrollment Network (OPEN) is a web-based registration system available on a 24/7 basis. OPEN is integrated with CTSU regulatory and roster data and with the LPOs registration/randomization systems or the Theradex Interactive Web Response System (IWRS) for retrieval of patient registration/randomization assignment. OPEN or IWRS will populate the patient enrollment data in NCI's clinical data management system, Medidata Rave.

Requirements for OPEN access:

- A valid CTEP-IAM account.
- To perform enrollments or request slot reservations: Must be on an LPO roster, ETCTN corresponding roster, or PO roster with the role of Registrar. Registrars must hold a minimum of an Associate Plus (AP) registration type.
- If a DTL is required for the study, the registrar must hold the OPEN Registrar task on the DTL for the site.
- Have an approved site registration for the protocol prior to patient enrollment.

To assign an Investigator (IVR) or Non-Physician Investigator (NPIVR) as the treating, crediting, consenting, drug shipment (IVR only), or receiving investigator for a patient transfer in OPEN, the IVR or NPIVR must list the IRB number used on the site's IRB approval on their Form FDA 1572 in RCR. If a DTL is required for the study, the IVR or NPIVR must be assigned the appropriate OPEN-related tasks on the DTL.

Prior to accessing OPEN, site staff should verify the following:

- Patient has met all eligibility criteria within the protocol stated timeframes, and
- All patients have signed an appropriate consent form and Health Insurance Portability and Accountability Act (HIPAA) authorization form (if applicable).

Note: The OPEN system will provide the site with a printable confirmation of registration and treatment information. IWRS system also sends an email confirmation of the registration. You may print this confirmation for your records.

Access OPEN at https://open.ctsu.org or from the OPEN link on the CTSU members' website. Further instructional information is in the OPEN section of the CTSU website at https://www.ctsu.org or https://open.ctsu.org. For any additional questions, contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

Patient enrollment will be facilitated using the Slot Reservation System in conjunction with the registration system in OPEN. Prior to discussing protocol entry with the patient, all site staff must use the CTSU OPEN Slot Reservation System or the IWRS Slot Reservation System to ensure that a slot on the protocol is available to the patient. Once a slot reservation confirmation is obtained, site staff may then proceed to enroll the patient to this study.

4.3.2 Special Instructions for Patient Enrollment

- All biospecimens collected for this trial must be submitted using the ETCTN Specimen Tracking System (STS) unless otherwise noted.
- The system is accessed through Rave user roles: "Rave CRA" and "Rave CRA (Labadmin)" for data entry at the treating institutions and "Biorepository" for users receiving the specimens for processing and storage at reference labs and the NCI Early-Phase and Experimental Clinical Trials Biospecimen Bank (EET Biobank, formerly known as the ETCTN Biorepository).

- Please refer to the Medidata Account Activation and Study Invitation Acceptance link on the CTSU website in the Data Management section under the Rave Home tab and then under Rave Resource Materials.
- **Important: Failure to complete required fields in STS may result in a delay in sample processing.** Any case reimbursements associated with sample submissions will not be credited if samples requiring STS submission are not logged into STS.

Detailed instructions on use of the STS can be found in Section 5.3.

4.3.3 OPEN/IWRS Questions?

Further instructional information on OPEN is provided on the OPEN link of the CTSU website at https://www.ctsu.org or at https://open.ctsu.org. For any additional questions contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

Theradex has developed a Slot Reservations and Cohort Management User Guide, which is available on the Theradex website: http://www.theradex.com/clinicalTechnologies/?National-Cancer-Institute-NCI-11. This link to the Theradex website is also on the CTSU website OPEN tab. For questions about the use of IWRS for slot reservations, contact the Theradex Helpdesk at 855-828-6113 or Theradex main number 609-799-7580; CTMSSupport@theradex.com.

4.4 General Guidelines

Following registration, patients should begin protocol treatment within 21 days. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Study Coordinator should be notified of cancellations as soon as possible.

5. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

5.1 Summary Table for Specimen Collection

Note: The Phase 1b portion of the trial is now closed. Moving forward, all new patients will be enrolled in the Phase II portion of the trial as per Amendment #04 (July 13, 2022).

Time Point	Specimen	Send Specimens To:
Baseline/Screening		
	• 200 million live bone marrow mononuclear cells (mandatory)[1,2] • Unstained pathology slide – smear of bone marrow aspirate (mandatory) • Unstained pathology slide - cytospin of bone marrow mononuclear cells (optional)	EET Biobank – Leukemia Division
C1D5 (Phase 1b and Phase 2 Venetoclax + ASTX727 Arm Only)		

Pre-venetoclax, 1, 2, 4, 7 h (after dose)	• 6 mL Whole Blood in ETDA tubes processed for plasma and frozen (mandatory)	OSUCCC PhASR for ChOP-KC
C1D8 (Phase 1b and Phase 2 Venetoclax + ASTX727 Arm Only)		
	• 5 million live bone marrow mononuclear cells (mandatory)[1,2] • Unstained pathology slide – smear of bone marrow aspirate (mandatory) • Unstained pathology slide- cytospin of bone marrow mononuclear cells (optional)	EET Biobank – Leukemia Division
C1D22 ± 2 days (Phase 1b and Phase 2 Venetoclax + ASTX727 Arm Only)		
Pre-venetoclax, 1, 2, 4, 7 h (after dose)	• 6 mL Peripheral Blood in ETDA tubes processed for plasma and frozen (mandatory)	OSUCCC PhASR for ChOP-KC
Progression		
	• 200 million live bone marrow mononuclear cells (optional)[1,2] • Unstained pathology slide – smear of bone marrow aspirate (optional) • Unstained pathology slide- cytospin of bone marrow mononuclear cells (optional)	EET Biobank – Leukemia Division
[1]≥15mL from bone marrow aspirate (not counting the ACD in the tube) is the preferred volume requested per biopsy time point irrespective of actual cell number. **[2]For bone marrow aspirate or biopsy, the corresponding bone marrow report must be submitted uploaded to Rave and sent with the specimen to the EET Biobank – Leukemia Division. If the report is not available at time of shipment, then send it to the Biobank and upload to Rave as soon as it becomes available.**		

5.2 Specimen Procurement Kits and Scheduling

5.2.1 Specimen Procurement Kits

Kits for the collection and shipment of specimens to the EET Biobank – Leukemia Division are not provided for this study. Sites must provide collection and shipping supplies.

5.2.2 Scheduling of Specimen Collections to the EET Biobank – Leukemia Division

Please adhere to the following guidelines when scheduling procedures to collect samples:

- Frozen bone marrow specimens and plasma may be collected and shipped Monday through Thursday.

5.3 Specimen Tracking System Instructions

5.3.1 Specimen Tracking System Overview and Enrollment Instructions

For the ETCTN STS, the following information will be requested:

- Protocol Number
- Investigator Identification
  - Institution and affiliate name
  - Investigator's name
- Eligibility Verification: Patients must meet all the eligibility requirements listed in Section 3.
- Additional Requirements:
  - Patients must provide a signed and dated, written informed consent form.

Upon enrolling a patient, IWRS will communicate with OPEN, assigning two separate and unique identification numbers to the patient, a Universal patient ID (UPID) and a Treatment patient ID. The UPID is associated with the patient and used each and every time the patient engages with the portion of this or any other protocol that uses the ETCTN Specimen Tracking System. The UPID contains no information or link to the treatment protocol. IWRS will maintain an association between the UPID for ETCTN biobanking and molecular characterization and any treatment protocols the patient participates in, thereby allowing analysis of the molecular characterization results with the clinical data.

Immediately following enrollment, the institutional anatomical pathology report for the diagnosis under which the patient is being enrolled must be uploaded into Rave. The report must include the surgical pathology ID (SPID), collection date, block number, and the IWRS-assigned UPID and patient study ID for this trial. For newly acquired biopsies without a corresponding pathology report, the radiology and operative report(s) must also be uploaded into Rave, when available. **Important: Remove any personally identifying information, including, but not limited to, the patient's name, date of birth, initials, medical record number, and patient contact information from the institutional pathology report prior to submission.**

Additionally, please note that the STS software creates pop-up windows when reports are generated, so you will need to enable pop-ups within your web browser while using the software.

For questions regarding the Specimen Tracking System, please contact STS Support at STS.Support@theradex.com.

The Shipping List report **must** be included with all sample submissions.

5.3.2 Specimen Labeling

5.3.2.1 Bone Marrow Specimen Labels

Include the following on bone marrow specimens:

- Patient Study ID
- Universal Patient ID (UPID)

- Specimen ID (automatically generated by Rave)
- Time point
- Specimen type (*e.g.*, blood, serum, bone marrow)
- Laterality (bone marrow) (e.g. Right (R) or Left (L) Side if both submitted) – to be added by hand)
- Collection date and time (to be added by hand)
- Post-processing: # of live cells per tube

5.3.2.2 Blood Specimen Labels

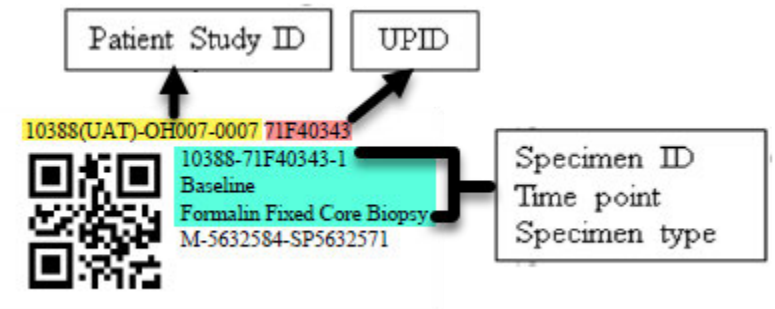
Include the following on blood specimens (including frozen, processed blood products – like serum):

- Patient Study ID
- Universal Patient ID (UPID)
- Specimen ID (automatically generated by Rave)
- Time point
- Specimen type (*e.g.*, serum, plasma)
- Collection date (to be added by hand)

5.3.2.3 Example of Specimen Label Generated by STS

STS includes a label printing facility, accessed via the Print Label CRF in the All Specimens folder. A generated PDF is emailed to the user as a result of saving that form.

The following image is an example of a tissue specimen label printed on a label that is 0.5" high and 1.28" wide.



The QR code in the above example is for the Specimen ID shown on the second line.

Labels may be printed on a special purpose label printer, one label at a time, or on a standard laser printer, multiple labels per page. Theradex recommends the use of these low temperature waterproof labels for standard laser printers: https://www.labtag.com/shop/product/cryo-laser-labels-1-28-x-0-5-cl-23-colors-available/

The last line item on the label includes the following data points joined together:

1. Tissue only: Primary (P), Metastatic (M), Normal (N) tissue indicated at the beginning of the specimen ID; this field is blank if not relevant (*e.g.*, for blood)
2. Block ID or blank if not relevant
3. SPID (Surgical Pathology ID) or blank if none
4. An optional alpha-numeric code that is protocol specific and is only included if the protocol requires an additional special code classification

Space is provided at the bottom of the label for the handwritten date and optional time. The last line on the example label is for the handwritten date and optional time.

5.3.3 Overview of Process at Treating Site

5.3.3.1 OPEN Registration

All registrations will be performed using the Oncology Patient Enrollment Network (OPEN) system. OPEN communicates automatically with the Interactive Web Response System (IWRS) which handles identifier assignments, any study randomization, and any prescribed slot assignments. If specimen analysis is required to determine eligibility, the protocol will be setup with multi-step registration.

Registration without eligibility specimen analysis:

1. Site enters registration data into OPEN during one or more steps.
2. IWRS receives data from OPEN, generates the Patient Study ID and the Universal Patient ID, both of which are sent back to OPEN.
3. IWRS sends all applicable registration data directly to Rave at the end of the final registration step.

5.3.3.2 Rave Specimen Tracking Process Steps

Step 0: Log into Rave via your CTEP-IAM account, then navigate to the appropriate participant.

Step 1: Complete the **Histology and Disease** form (but do not upload reports until a specimen label can be applied to them) and the Baseline forms regarding **Prior Therapies**. Enter the initial clinical specimen data:

- **Specimen Tracking Enrollment** CRF: Enter Time Point, Specimen Category, Specimen Type, Block number, Tissue type, Surgical Path ID, and number of labels needed (include extra labels to apply to reports to be uploaded). CRF generates unique Specimen ID.

Step 2: Print labels using the **Print Labels** CRF located in the All Specimens folder, then collect specimen.

- Label specimen containers and write collection date and time on each label. After collection, store labeled specimens as described in Section 5.4.2.
- Apply an extra specimen label to *each* report before scanning. Return to the **Histology and Disease** form to upload any initial Pathology, Radiology, Bone Marrow, Molecular Reports (up to 4), and Surgical (or Operative) reports. Return to **Specimen Tracking Enrollment** CRF to upload any molecular report (one per specimen) and/or specimen specific pathology or related report (one per specimen) and Tissue Biopsy Verification form (when applicable). Uploaded reports should have protected health information (PHI) data, like name, date of birth, mailing address, medical record number or social security number (SSN), redacted. **Do not redact SPID, block number, diagnosis or relevant dates (such as collection date), and include the UPID and patient study ID on each document** (either by adding a label or hand writing).

Step 3: Complete specimen data entry.

- **Specimen Transmittal** Form: Enter collection date and time and other required specimen details.

Step 4: When ready to ship, enter shipment information.

- **Shipping Status** CRF: Enter tracking number, your contact information, recipient, number of sample containers and ship date once for the first specimen in a shipment.
- **Copy Shipping** CRF: In the specimen folders for additional specimens (if any) that will be shipped with the initial specimen, please use the **Copy Shipping** form to derive common data into additional **Shipping Status** forms. A few unique fields will still need to be entered in **Shipping Status.**

Step 5: Print shipping list report and prepare to ship.

- Shipping List report is available at the site level.
- Print two copies of the shipping list, one to provide in the box, the other for your own records.
- Print pathology or other required reports to include in the box. Be sure the printed copy includes the specimen label.

Step 6: Send email notification.

- For only one of the specimens in the shipment, click "Send Email Alert" checkbox on the **Shipping Status** CRF to email recipient.

Step 7: Ship the specimen(s).

Step 8: Monitor the Receiving Status form located in each specimen folder for acknowledgment of receipt and adequacy.

5.4 Specimen Collection

5.4.1 Collection of Bone Marrow

Collect bone marrow aspirate in an acid citrate dextrose (ACD- yellow top) 7mL collection tube.

Bone marrow samples may be held for no longer than 24 hours at 4°C before processing and may be processed in batches.

Bone marrow will be processed and frozen according to the procedures described in Appendix H.

5.4.2 Collection of Blood in EDTA Tubes for Plasma Processing (See Appendix I)

5.5 Shipping Specimens from Clinical Site to the EET Biobank – Leukemia Division

5.5.1 General Shipping Information

Batch ship cryopreserved bone marrow mononuclear cells and frozen plasma on dry ice via FedEx Priority overnight. Batches may include specimens from up to 5 patients.

5.5.1.1 Required Forms for Specimen Submissions:

Each document submitted with the specimen must be labeled with a label printed from the STS, or the Universal ID and Patient Study ID.

Tissue	Required Forms
Bone Marrow Aspirate Mononuclear Cells	1.Shipping List 2.Corresponding Bone Marrow Report (once available)
Plasma	1. Shipping List

5.5.2 Specimen Shipping Instructions

Frozen bone marrow mononuclear cells and plasma may be shipped on Monday through Thursday.

5.5.2.1 Shipping of Glass Slides Using Institutional Supplies

1. Before packaging smear and cytopsin slides, verify that each specimen is labeled according to Section 5.3.2.1.
2. Glass slides must be placed in plastic slide holders. Place tissue paper on top of the separated slides prior to closing the slide holder to reduce slide movement during shipment.
3. Place the slides in a reinforced cardboard shipping box with appropriate packaging filler to minimize movement of specimens within the shipping box.
4. Include a copy of the forms listed above and a shipping manifest from the Specimen Tracking System with each shipment.

5. Please include a cold pack when shipping on hot days and extra insulation on cold days.
6. Ship specimens to the address listed below. FedEx Priority Overnight is strongly recommended to prevent delays in package receipt.

5.5.2.2 Shipping Frozen Specimens Using Supplies Provided by the Institution

1. Before packaging specimens, verify that each specimen is labeled according to the instructions in 5.3.2 and that lids of all primary receptacles containing liquid are tightly sealed.
2. Place the specimens in zip-lock bags. Use a separate zip-lock bag for each specimen type and time point.
3. Place the zip-lock bags in a biohazard envelope containing absorbent material. Expel as much air as possible and seal securely.
4. Put the secondary envelope into a Tyvek envelope. Expel as much air as possible and seal securely.
5. Place frozen specimens into the insulated shipping container with dry ice. Layer the bottom of the container with dry ice until it is approximately one-third full. Place the frozen specimens on top of the dry ice. Cover the specimens with the dry ice until the container is almost completely full. When packaging specimens, ensure that you leave enough room to include at least 5 pounds of dry ice in the shipment. If more than 20 cryovials are included in the shipment, then additional dry ice will be needed.
6. Insert a copy of the shipping list and corresponding bone marrow report (if available) into a plastic bag and place in the shipping container.
7. Close the shipping container and tape it shut with durable sealing tape. Do not completely seal the container.
8. Complete a FedEx air bill and attach to top of shipping container.
9. Complete a dry ice label.
10. Attach the dry ice label and an Exempt Human Specimen sticker to the side of the shipping container.
11. Ship specimens via overnight courier to the address below. FedEx Priority Overnight is strongly recommended to prevent delays in package receipt.

5.5.3 Shipping Address

Ship to the address below. Do not ship specimens the day before a holiday.

EET Biobank – Leukemia Division
Nationwide Children's Hospital
700 Children's Drive, C0825
Columbus, Ohio 43205
PH: (614) 722-3270
FAX: (614) 722-2856
Email**:** BPCMGL@nationwidechildrens.org
FedEx Priority Overnight service is very strongly preferred.

NOTE: The EET Biobank FedEx Account will not be provided to submitting institutions.

There is no central Courier account for this study. Sites are responsible for the cost of shipments to the EET Biobank.

5.5.4 Contact Information for Assistance

For all queries, please use the contact information below:

EET Biobank – Leukemia Division
Phone: (614) 722-3270
E-mail: BPCMGL@nationwidechildrens.org

5.6 Biomarker Plan

List of Biomarker Assays in Order of Priority

Note for participating sites: Please see Section 5.1 for details on specimens to collect. The specimens tested are not always the same specimens that are submitted by the site, as processing of blood and tissue will occur at the Biobank prior to testing. The Phase 1b portion of the trial is now closed. Moving forward, all new patients will be enrolled in the Phase II portion of the trial as per Amendment #04 (July 13, 2022).

Priority	Biomarker Name	Assay (CLIA: Y/N)	Use in the Trial and Purpose	Specimens Tested	Collection Time Points	Mandatory or Optional	Assay Laboratory and Lab PI
Tissue-based Biomarkers							
1	DNA variants of Leukemic Initiating Cells (LIC)	FACS and deep DNA sequencing NGS/Illumina HiSeq 2500 504-gene custom panel CLIA: N	Integrated To identify biomarkers of response/resistance by identifying primitive clonal cells pre and post treatment and correlate with response and transcriptomic signatures	Bone marrow mononuclear cells Frozen	Baseline/Screening and Cycle 1 Day 8 (Phase 1b and Phase 2) Venetoclax+ASTX727 Arm only)	M	Verma Laboratory, Albert Einstein Cancer Center Amit Verma Amit.verma@einsteinmed.org Steidl Laboratory, Albert Einstein College of Medicine Ulrich Steidl ulrich.steidl@einsteinmed.org
2	LIC Transcriptome signatures	Single cell RNA sequencing NGS: Whole transcriptome shotgun sequencing (WTSS)/ RNAseq CLIA: N	Exploratory To identify biomarkers of response/resistance through transcriptomic signatures in responders *vs.* non-responders	Bone marrow mononuclear cells Frozen Specimens are collected as part of collection for DNA sequencing assay	Baseline/Screening and Cycle1 Day 8 (Phase 1b and Phase 2) Venetoclax+ASTX727 Arm only)	M	Verma Laboratory Albert Einstein Cancer Center Amit Verma Amit.verma@einsteinmed.org
3	Morphology/ Maturation and drug sensitivity	Flow based drug screen CLIA: N	Exploratory To identify biomarkers of response- assess drug sensitivity of each cell type and stage of disease maturation	Bone marrow mononuclear cells Frozen	Baseline/Screening	M	Savona Laboratory Vanderbilt University Medical Center Michael Savona michael.savona@vumc.org

Priority	Biomarker Name	Assay (CLIA: Y/N)	Use in the Trial and Purpose	Specimens Tested	Collection Time Points	Mandatory or Optional	Assay Laboratory and Lab PI
4	Alternative therapy response	PDX Co-clinical trial CLIA:N	Exploratory To determine if treatment failure is a function of therapy choice and if treatment failure results in resistance to the alternative therapy	Bone marrow mononuclear cells Frozen	Baseline/Screening and progression	O	Savona Laboratory Vanderbilt University Medical Center Michael Savona michael.savona@vumc.org
Blood-based Biomarkers							
1	Venetoclax PK	LS-MS/MS CLIA:N	Exploratory Assessment of exposure-toxicity/efficacy associations during AML therapy	Plasma	Phase 1b and Phase 2 Venetoclax+ASTX727 Arm only: C1D5: Pre-venetoclax, 1, 2, 4, 7 h (after dose) C1D22 ±2 days: Pre-venetoclax, 1, 2, 4, 7 h (after dose)	M	Baker Laboratory OSUCCC PhASR for ChOP-KC Sharyn Baker baker.2480@osu.edu

5.7 Integrated Correlative Studies

5.7.1 DNA variants of Leukemic Initiating Cells (LIC)

5.7.1.1 Specimen Receipt and Processing at the EET Biobank – Leukemia Division

The EET Biobank – Leukemia Division will receive cryopreserved bone marrow mononuclear cells from participating sites. The EET Biobank – Leukemia Division will ship the frozen bone marrow to the site performing the correlative study. Samples should be sent from the EET Biobank – Leukemia Division as efficiently as possible after receipt and can be sent in batches to each participating laboratory at least every 12 weeks for analysis.

5.7.1.2 Site(s) Performing Correlative Study

Assays will be performed at the Verma Laboratory, Albert Einstein Cancer Center, under the supervision of Amit Verma and at the Steidl Laboratory, Albert Einstein College of Medicine, under the supervision of Ulrich Steidl, the Baker Laboratory at the Ohio State University Medical Center under the supervision of Sharyn Baker, and the Savona Laboratory under supervision of Michael Savona, Vanderbilt University Medical Center.

5.7.1.3 Shipment of specimens from the EET Biobank – Leukemia Division to Site Performing Correlative Study

Specimens will be shipped from the EET Biobank – Leukemia Division to:

Verma Laboratory, Albert Einstein Cancer Center
Albert Einstein College of Medicine - Research
1300 Morris Park Ave C302B, Bronx, NY 10464

5.7.1.4 Contact information for notification of specimen shipment

Amit Verma
amit.verma@einsteinmed.org

Ulrich Steidl
ulrich.steidl@einsteinmed.org

Sharyn Baker (c/o Kasey Hill)
phasr@osumc.edu

Michael Savona (c/o Melissa Fischer)

mel.steaplet@vumc.org

5.8 Exploratory/Ancillary Correlative Studies

5.8.1 LIC transcriptomic signatures

5.8.1.1 Specimen Receipt and Processing at the EET Biobank – Leukemia Division

The EET Biobank – Leukemia Division will receive cryopreserved bone marrow mononuclear cells from participating sites. The EET Biobank – Leukemia Division will ship the frozen bone marrow to the site performing the correlative study. Samples should be sent from the EET Biobank – Leukemia Division as efficiently as possible after receipt and can be sent in batches to each participating laboratory at least every 12 weeks for analysis.

5.8.1.2 Site(s) Performing Correlative Study

This assay will be performed at the Verma Laboratory, Albert Einstein Cancer Center, under the supervision of Amit Verma.

5.8.1.3 Shipment of specimens from the EET Biobank – Leukemia Division to Site Performing Correlative Study

Specimens will be shipped from the EET Biobank – Leukemia Division to:

Verma Laboratory, Albert Einstein Cancer Center
Albert Einstein College of Medicine - Research
1300 Morris Park Ave C302B, Bronx, NY 10464

5.8.1.4 Contact information for notification of specimen shipment

Amit Verma

Amit.verma@einsteinmed.org

Ulrich Steidl
ulrich.steidl@einsteinmed.org

5.8.2 Morphology/maturation and drug sensitivity

5.8.2.1 Specimen Receipt and Processing at the EET Biobank – Leukemia Division

The EET Biobank – Leukemia Division will receive cryopreserved bone marrow mononuclear cells from participating sites. The EET Biobank – Leukemia Division will ship the frozen bone marrow to the site performing the correlative study. Samples should be sent from the EET Biobank – Leukemia Division as efficiently as possible after receipt and can be sent in batches to each participating laboratory at least every 12 weeks for analysis.

5.8.2.2 Site(s) Performing Correlative Study

This assay will be performed at the Savona Laboratory, Vanderbilt University Medical Center, under the supervision of Michael Savona.

5.8.2.3 Shipment of specimens from the EET Biobank – Leukemia Division to Site Performing Correlative Study

Specimens will be shipped from the EET Biobank – Leukemia Division to:

Savona Laboratory (c/o Melissa Fischer)
Vanderbilt University Medical Center/Vanderbilt-Ingram Cancer Center
2220 Pierce Avenue
Preston Research Building, Suite 518
Nashville, TN 37232

5.8.2.4 Contact information for notification of specimen shipment

Michael Savona (c/o Melissa Fischer)
mel.steaplet@vumc.org

5.8.3 Alternative therapy response

5.8.3.1 Specimen Receipt and Processing at the EET Biobank – Leukemia Division

The EET Biobank – Leukemia Division will receive cryopreserved bone marrow mononuclear cells from participating sites. The EET Biobank – Leukemia Division will ship the frozen bone marrow to the site performing the correlative study. Samples should be sent from the EET Biobank – Leukemia Division as efficiently as possible after receipt and can be sent in batches to each participating laboratory at least every 12 weeks for analysis.

5.8.3.2 Site(s) Performing Correlative Study

This assay will be performed at the Savona Laboratory, Vanderbilt University Medical Center, under the supervision of Michael Savona.

5.8.3.3 Shipment of specimens from the EET Biobank – Leukemia Division to Site Performing Correlative Study

Specimens will be shipped from the EET Biobank – Leukemia Division to:

Savona Laboratory (c/o Melissa Fischer)
Vanderbilt University Medical Center/Vanderbilt-Ingram Cancer Center
2220 Pierce Avenue
Preston Research Building, Suite 518
Nashville, TN 37232

5.8.3.4 Contact information for notification of specimen shipment

Michael Savona (c/o Melissa Fischer)
mel.steaplet@vumc.org

5.8.4 Pharmacokinetic Studies

5.8.4.1 Specimen Receipt and Processing at the OSUCCC PhASR for ChOP-KC

The OSUCCC PhASR for ChOP-KC lab will receive cryopreserved plasma samples from participating sites (see Appendix I).

5.8.4.2 Site(s) Performing Correlative Study

This assay will be performed at the OSUCCC PhASR for ChOP-KC, under the supervision of Sharyn Baker.

Shipment of specimens to the OSUCCC PhASR for ChOP-KC Specimens will be shipped to the OSUCCC PhASR for ChOP-KC:

The OSUCCC PhASR
Attn: Kasey Hill, Ph.D. (Venetoclax Samples)
441 Biomedical Research Tower
460 West 12th Avenue
Columbus, OH 43210
Phone: (614) 688-0578
Fax: (614) 292-7766
Sharyn Baker (c/o Kasey Hill)
phasr@osumc.edu

6. TREATMENT PLAN

6.1 Agent Administration

For phase 1b treatment and phase 2 randomized intervention arm, it is encouraged that venetoclax + ASTX727 treatment be administered inpatient during the first cycle (during the ramp-up for venetoclax + ASTX727) to monitor for and manage potential TLS. In the phase 2 portion of the study, per the dosing schema, patients will be randomized to the venetoclax +ASTX727 Arm or 7+3 Arm. For 7+3 Arm of randomized trial, patients will be treated as inpatients and per institutional SOP. For the experimental venetoclax + ASTX727 arm, investigators will be encouraged to treat patients as inpatient during the first cycle (during ramp up to monitor for and manage potential TLS) and thereafter may be treated as inpatients or

outpatients per investigator decision based on patient care requirements and as per institutional SOP for low-intensity regimens. Reported adverse events and potential risks are described in Section 10. Dose de-escalation schema and appropriate dose modifications are described in Section 7. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

Patients enrolled in phase 1b/safety run-in phase will receive a combination of venetoclax and ASTX727; To determine the recommended phase 2 dose of venetoclax and ASTX727 combination, a 3+3 dose de-escalation design will be employed in the phase 1b part of the study. The 3+3 dose de-escalation plan is described in further detail under Section 6.2. Note: The Phase 1b portion of the trial is now closed. Moving forward, all new patients will be enrolled in the Phase II portion of the trial as per Amendment #04 (July 13, 2022).

Dose de-escalation schedule for phase 1b

Dose schedule for phase 1b			
Dose Level	**Dose**		**Cycle Length**
	ASTX727, PO	**Venetoclax, PO****	
*Level 1	1 FDC Tablet[#], QD D1-5	400mg, QD D1-28	28 days
Level -1	1 FDC Tablet, QD D1-4	400mg, QD D1-28	
Level -2	1 FDC Tablet, QD D1-4	400mg, QD D1-21	
*Starting dose level. [#]ASTX727 is a Fixed Dose Combination (FDC) tablet containing 35mg decitabine + 100mg cedazuridine **Follow ramp up dosing scheme in Section 6.1.1.2. PO = Per Oral, QD = Once a Day			

Regimen for the phase 1b trial

Regimen Description					
Agent	**Premedications ; Precautions**	**Dose**	**Route**	**Schedule#**	**Cycle Length**
Venetoclax	Adequate hydration, anti-hyperuricemic agents (eg allopurinol or feboxustat) >48 hours prior to starting venetoclax. Monitor blood	400 mg (refer to ramp up dosing scheme in Section 6.1.1.2)	PO	**QD	28 days (4 weeks)

	chemistries for TLS at pre-dose, 6 to 8 hours after each new dose during ramp-up, and 24 hours after reaching final dose				
ASTX727	Patients should be pre-medicated for nausea and vomiting. Clear liquids only 2 hours pre- and 2 hours post dose	1 FDC Tablet	PO	**QD	
The patient will be requested to maintain a medication diary of each dose of medication. The medication diary will be returned to clinic staff at the end of each course. **Schedule as appropriate for assigned dose level. #Follow the timing and order of administration described in Section 6.1.2.					

Regimen for phase 2 (Venetoclax+ASTX727 Arms):

Regimen Description					
Agent	**Premedications ; Precautions**	**Dose**	**Route**	**Schedule#**	**Cycle Length***
Venetoclax	Adequate hydration, anti-hyperuricemic agents (eg allopurinol or feboxustat) >48 hours prior to starting venetoclax. Monitor blood chemistries for TLS at pre-dose, 6 to 8 hours after each new dose during ramp-up, and 24 hours after reaching final	400mg (refer to ramp up dosing scheme in Section 6.1.1.2)	PO	QD	28 days (4 weeks)

	dose				
ASTX727	Patients should be pre-medicated for nausea and vomiting and allopurinol/IVF for TLS precautions. Clear liquids only 2 hours pre- and 2 hours post dose	1 FDC Tablet	PO	Days 1-5	
Notes: *Treatment will continue for a maximum of 12 cycles. The patient will be requested to maintain a medication diary of each dose of medication. The medication diary will be returned to clinic staff at the end of each course. #Follow the timing and order of administration described in Section 6.1.2.					

Regimen for phase 2 (7+3 Control Arm)**

Induction Therapy Regimen Description					
Agent	**Premedications ; Precautions**	**Dose***	**Route**	**Schedule**	**Cycle# Length**
Cytarabine	Premedications/ precautions as per standard local procedures. Ondansetron 16mg q24 hours (required D1-3; optional D4-7)	100 mg/m^2 Reconstitution and diluents as per standard local procedures/ per package insert. Refer to Section 8.2.1 for additional information.	Continuous IV	Days 1-7	28 days (4 weeks)
Daunorubicin	Premedications/ precautions as per local standard procedures.	60 mg/m^2 Daunorubicin on Days 1, 2, and 3		Days 1-3	28days (4 weeks)

Induction Therapy Regimen Description					
Agent	**Premedications ; Precautions**	**Dose***	**Route**	**Schedule**	**Cycle[#] Length**
	Ondansetron 16mg q24 hours and Dexamethasone 8mg q12 hours during days of daunorubicin administration.	Reconstitution and diluents as per standard local procedures/ per package insert. Refer to Sections 8.2.2 and 8.2.3 for additional information.			
** This regimen should be used as general guidance. Otherwise, it is permissible to follow institutional standards. *Dose rounding per institutional guidelines is allowed. # Patients with residual leukemia in bone marrow on Day 14-21 bone marrow biopsy will be allowed one additional reinduction chemotherapy cycle. Residual leukemia would include presence of >10% blasts in the mid-cycle bone marrow, with >10% cellularity. If increased blasts are present with less than 10% cellularity, the PI must be contacted prior initiating reinduction." Re-induction chemotherapy cycle may be offered (cytarabine at 100 mg/m^2/day continuous IV on Days 1-5 and daunorubicin 60 mg/m^2/day IV on Days 1-2). Midcycle bone marrow biopsy (Day 14-21) will be done per SOC but is required if additional treatment is given.					

6.1.1 CTEP Agents

6.1.1.1 ASTX727

ASTX727 is an orally administered fixed-dose combination of the cytidine deaminase inhibitor (CDAi) cedazuridine (100mg) and the DNMTi decitabine (35mg). ASTX727 is available only in fixed dose 100mg/35mg tablets and is not currently FDA-approved for AML. ASTX727 will be supplied by Astex and distributed by PMB through the CTEP CRADA. Patients will be given a supply of tablets to be taken at home for non-clinic dosing days.

ASTX727 should be taken approximately at the same time on an empty stomach (morning before breakfast). Do not eat, drink milk or alcohol 2 hours before and 2 hours after taking ASTX727. Clear liquids such as water, black coffee, or tea are allowed. Take whole tablets with 8 oz. (240 mL) of water; do not crush, cut, or chew the tablet.

Missed dose within 12 hours of the scheduled time can be made up. Patient needs to take as soon as possible and resume the next day on schedule. If the patient fails to make up the dose within 12 hours, the dose should be resumed at the scheduled time the next day.

If a patient vomits within one hour after dosing, the doses of ASTX727 should be re-administered. Antiemetics should be administered prior to subsequent doses.

Doses of ASTX727 that are omitted for AEs or for any other reason should not be replaced or made up at the end of the dosing period.

Patients will receive venetoclax and ASTX727 until progression of disease, unacceptable toxicity, revocation of consent, or for up to 12 months. After 12 months, patients may continue on therapy for as long as they continue to respond. On days involving dosing of both ASTX727 and venetoclax, the order of ASTX727/venetoclax administration is not specified – *i.e*. either oral drug, ASTX727 or venetoclax, may be taken before or after the other drug. However, if patient experience nausea from ASTX727 despite antinausea prophylaxis, patient should receive next dose of venetoclax >2 hours prior to next dose of ASTX727.

The following ASTX727 dose schedule will be evaluated: 1 tablet of a Fixed Dose Combination (FDC) of 35mg decitabine + 100mg cedazuridine administered daily x 5 days.

6.1.1.2 Venetoclax

Venetoclax is an FDA-approved small molecule inhibitor of B-cell lymphoma 2 (BCL2) used to treat chronic lymphocytic leukemia and in combination with low dose cytarabine (LDAC) or DNA methyltransferase inhibitor (DNMTi) therapy for acute myeloid leukemia (AML) in the elderly. Venetoclax is supplied 100mg tablets and is not FDA-approved for AML in patients under 65 years of age.

Due to possible CYP3A mediated metabolic interaction certain food items (*e.g*., grapefruit and Seville oranges) should not be consumed during treatment with venetoclax except as noted in the protocol (see Section 6.3.2).

Venetoclax should be taken with food and water at the same time each day (within 30 minutes of a meal). The tablets should be swallowed whole (not crushed or broken). If possible, patients should drink 6-8 glasses of water daily for two days before initiating therapy with venetoclax.

Venetoclax Ramp Up Schedule:

Each patient is scheduled to receive oral venetoclax on a continuous once-a-day (Q.D.) dosing schedule. Administer the venetoclax dose according to a daily ramp-up schedule over 3 days in Cycle 1 to the recommended daily dose of 400 mg as shown below.

Cycle 1:

C1D1: Venetoclax 100 mg PO daily with breakfast at least 2 hours after ASTX727.

C1D2: Venetoclax 200 mg PO daily with breakfast at least 2 hours after ASTX727.

C1D3-5: Venetoclax 400 mg PO daily with breakfast at least 2 hours after ASTX727.

C1D6-28: Venetoclax 400 mg PO daily with breakfast at the same time each day.

Cycle 2+

D1-5: Venetoclax 400 mg PO daily with breakfast at least 2 hours after ASTX727.

Days 6-28: Venetoclax 400 mg PO daily with breakfast at the same time each day.

In the absence of unacceptable adverse events requiring dose reduction of venetoclax, it is intended that all patients will receive venetoclax by the same dose and schedule. Patients will take venetoclax tablets with a meal and water at approximately the same time each day. Venetoclax tablets should be swallowed whole and not chewed, crushed, or broken prior to swallowing.

If the patient misses a dose of venetoclax within 8 hours of the time it is usually taken, the patient should take the missed dose as soon as possible and resume the normal daily dosing schedule. If a patient misses a dose by more than 8 hours, the patient should not take the missed dose and should resume the usual dosing schedule the next day. (Any additional instructions from the clinical team should be followed by the patient – *e.g*. dosing with respect to potential timing of biospecimen blood draws.)

If a patient vomits within one hour after dosing, the dose should be re-administered. If the patient vomits after 1 hour following dosing, no additional dose should be taken that day. The next prescribed dose should be taken at the usual time.

The following Venetoclax dose schedule will be evaluated: 400mg administered daily for the first 28 day cycle. For Cycle 1, there will be a 3-day dose ramp-up: C1D1 100mg, C1D2 200mg, C1D3-21 OR C1D3-28 400mg.

6.1.2 ASTX727 and Venetoclax Dosing Schedules

Cycle 1:

C1D1: ASTX727 given orally (PO) daily on an empty stomach (morning before breakfast) with no food for 2 hours before and at least 2 hours after administration; venetoclax 100 mg PO daily with breakfast at least 2 hours after ASTX727.

C1D2: ASTX727 PO daily on an empty stomach (morning before breakfast) with no food for 2

hours before and at least 2 hours after administration; venetoclax 200 mg PO daily with breakfast at least 2 hours after ASTX727.

C1D3-5: ASTX727 PO daily on an empty stomach (morning before breakfast) with no food for 2 hours before and at least 2 hours after administration; venetoclax 400 mg PO daily with breakfast at least 2 hours after ASTX727.

C1D6-28: venetoclax 400 mg PO daily with breakfast at the same time each day.

Cycle 2+:

D1-5: ASTX727 PO daily on an empty stomach (morning before breakfast) with no food for 2 hours before and at least 2 hours after administration; venetoclax 400 mg PO daily with breakfast at least 2 hours after ASTX727.

Days 6-28: venetoclax 400 mg PO daily with breakfast at the same time each day.

Venetoclax+ASTX727 Dosing Schedule, Cycle 1

DOSE De-ESCALATION			CYCLE 1																										
				Week 1							Week 2							Week 3							Week 4						
Day			-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Anti-TLS hydration/prophylaxis (oral / IVF) & Laboratory studies			X	X	X	X	X	X	X			X							X							X					
Anti-emesis (e.g. oral ondansetron)					X	X	X	X	X																						
ASTX727 (oral)#	Dose Levels	1			1 FDC Tablet	1 FDC Tablet	1 FDC Tablet	1 FDC Tablet	1 FDC Tablet																							
		-1			1 FDC Tablet	1 FDC Tablet	1 FDC Tablet	1 FDC Tablet																								
		-2			1 FDC Tablet	1 FDC Tablet	1 FDC Tablet	1 FDC Tablet																								

| | | |

		-1			1 FDC Tablet	1 FDC Tablet	1 FDC Tablet	1 FDC Tablet																													
		-2			1 FDC Tablet	1 FDC Tablet	1 FDC Tablet	1 FDC Tablet																													
Venetoclax (oral)	Dose Levels	1			400mg	400mg	400mg	400mg	400mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg
		-1			400mg	400mg	400mg	400mg	400mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg
		-2			400mg	400mg	400mg	400mg	400mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg							

[#]ASTX727 is a Fixed Dose Combination (FDC) tablet containing 35mg decitabine + 100mg cedazuridine

6.1.3 Other Agent(s)

6.1.3.1 Daunorubicin and Cytarabine

Supportive care for both cytarabine and daunorubicin involves administering oral ondansetron 16 mg every 24 hours as well as oral dexamethasone 8 mg every 12 hours for the first 3 days of treatment. Further supportive care with oral ondansetron 16 mg for days 4-7 of cytarabine treatment is optional based on the treating physician's discretion. Antimicrobial prophylaxis can be given according to institutional standard of practice but growth factor support is only allowed after conferring with the PI. For other drugs, refer to individual product US package inserts. Patients should receive all necessary supportive care, including blood product transfusions, and symptom control.

Daunorubicin should be prepared by injecting the calculated patient dose into 50-100 mL of 0.9% (normal) saline, and should be administered over 10-30 minutes in a free-flowing IV. The IV push route is not recommended, but may be used as per institutional standards.

Cytarabine should be prepared by diluting the calculated patient daily (24 hour) dose in a 1,000 mL bag of 0.9% (normal) saline and administered by continuous IV infusion at a rate sufficient to achieve 100 mg/m^2 in 24 hours.

6.1.2.2 Antifungals

Antifungal prophylaxis is commonly administered to patients with AML receiving induction therapy given the prolonged neutropenia introduced with cytotoxic chemotherapy. While this is less of a concern with new diagnosis AML (than with chronically immunosuppressed refractory AML), neutropenia is commonly seen with the use of venetoclax and DNMTi therapy. However, given drug-drug interactions between venetoclax and strong or moderate inhibitors of CYP3A, caution needs to be exhibited with use of these agents. **After C1D14,** prophylactic antifungal therapy with strong or moderate inhibitors of CYP3A is permitted as per institutional guidelines if given consistent with the venetoclax dose modification guidelines provided in Section 6.3.2.1.

6.2 Definition of Dose-Limiting Toxicity

Management and dose modifications associated with the above adverse events are outlined in Section 7.

A dose-limiting toxicity (DLT) is defined as any of the following that occurs up to day 28 during the Dose De-Escalation Phase in Phase 1b of the trial and is considered to be possibly related to study drug administration. With respect to excess hematologic toxicity, at least 42 days are allowed for hematologic recovery, so DLT evaluation must extend to ≤42 days before selecting the RP2D. This does not include toxicity with a clear alternative explanation (*i.e.*, disease progression) or transient (≤72 hours) abnormal laboratory values without associated clinically significant signs or symptoms based on investigator determination. All DLTs will be assessed by

the investigator using CTCAE v5.0 criteria.

In order to declare a dose level safe within the Phase 1b section of the study, subjects must receive requisite minimum criteria for dosing:

ASTX727: At least 4 of 5 scheduled oral doses of ASTX727 in DL 1, or 3 of 4 scheduled doses in DL -1 and DL-2 of the first cycle.
Venetoclax: The equivalent of ≥ 21 out of 28 doses of Venetoclax in DL1 or DL-1 during the first cycle or the equivalent of ≥ 16 out of 21 doses in DL-2.

Subjects receiving dose reductions (but not meeting DLT criteria) that result in cumulative dosing beneath the thresholds described above will not be considered evaluable for the purposes of determining the RP2D and will be replaced.

Non-hematologic

- Grade 3 nausea, vomiting, dehydration, diarrhea, or fatigue lasting > 3 days or requiring tube feeding, total parenteral nutrition, or requiring or prolonging hospitalization despite optimal supportive medications
- Any other Grade 3 non-hematological toxicity with the following exceptions:
  - Electrolyte abnormalities that resolve with or without intervention to < Grade 2 levels in < 72 hours.
  - Hair loss
  - ALT, AST, or alkaline phosphatase levels in the setting of baseline Grade 2 elevations from disease.
- Any Grade 4 non-hematologic toxicity.

Hematologic

- Neutropenia: Progression/development of Grade 4 neutropenia (**in the absence of residual disease**) during evaluability period and extending beyond D42

- Thrombocytopenia: Progression/development of Grade 4 thrombocytopenia ***(in the absence of residual disease***) during the evaluability period and extending beyond D42.

Exceptions

Adverse events that meet the above definitions but that are likely unrelated to study drug will not be considered to be DLTs.

For the purpose of ensuring an appropriate opportunity to determine the RP2D of Venetoclax in combination with ASTX727, a subject who is withdrawn from the study prior to completing the

DLT assessment window (i.e. first 28 days after initiating protocol-indicated treatment on Cycle 1, Day 1) for reasons other than a DLT will be considered not evaluable for DLT and will be replaced.

Additionally, over the first 28 days after initiating study therapy, if a Phase 1b patient discontinues study treatment for reasons clearly not related to protocol-indicated treatment (in the judgement of the Principal Investigator) and fails to complete at least 4 of 5 (or 3 of 4) scheduled oral doses of ASTX727 and at least 75% of scheduled oral doses of venetoclax (*i.e.* take at least 21 of 28 venetoclax doses), then that patient may be considered not evaluable for response to overall protocol-indicated treatment and may be replaced with a new patient.

Cohort review discussions will be held by the Principal Investigator, CTEP, and the drug suppliers to receive input from the investigators, in order to determine dose escalation, reductions, and expansions.

Management and dose modifications associated with the above adverse events are outlined in Section 7.

6.3 General Concomitant Medication and Supportive Care Guidelines

Because there is a potential for interaction of venetoclax with other concomitantly administered drugs, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The Principal Investigator should be alerted if the patient is taking any agent known to affect or with the potential for drug interactions. The study team should check a frequently-updated medical reference for a list of drugs to avoid or minimize use Appendix D (Patient Clinical Trial Wallet Card) should be provided to patients if available.

a. **Anti-TLS hydration/prophylaxis (oral / i.v.):** During cycle 1 of Venetoclax+ASTX727, TLS precautions will include daily visits with TLS laboratory studies and pre-emptive therapy with a uric acid reducing agent (i.e., allopurinol or febuxostat). Allopurinol to start ≥ 48h prior to C1D1 and continue through cycle 1 if no clinically significant TLS, and then at the discretion of investigator. In the event of inability to maintain copious fluid intake, intravenous fluids should be administered daily during C1. If there is clinical evidence of TLS in the opinion of the investigator(s), hospitalization should be considered.
b. **Antiemetic prophylaxis:** To minimize nausea, unless contraindicated, all patients in Phase 1b and Phase 2 must receive a 5-HT3 antagonist (e.g. ondansetron 4 mg or equivalent D1-5 for Venetoclax + ASTX727 arm, and 16mg or equivalent, D1-3 in the daunorubicin + cytarabine arm) starting at least 1 hour before patient's first dose. Alternative anti-emetic agent(s) may be used if the patient does not tolerate a 5-HT3 antagonist. After the patient completes the first 5 days of therapy with ASTX727, or the first 3 days of standard therapy, use of an anti-emetic agent is at the discretion of the patient's study physician.
c. **Antimicrobial prophylaxis**: Shall begin when ANC is below 500. Antibacterial, antiviral, and antifungal prophylaxis will be given in accordance with the protocol and consideration of CYP450-mediated concomitant medication risks as noted in protocol. For patients on venetoclax and ASTX727, antifungal treatment may not begin until after C1D14 (see Section 6.3.2.1).

d. **Hypokalemia risk**: Patients should be monitored for electrolytes twice during week 1 of Cycle 2 due to the high incidence of hypokalemia.

6.3.1 ASTX727

Drug-drug interaction studies have not been conducted with cedazuridine or decitabine. Nonclinical studies indicate that neither cedazuridine nor decitabine is likely to inhibit or induce major human cytochrome P450 enzymes. Thus, CYP450-mediated drug-drug interactions are not anticipated for cedazuridine or decitabine. Cedazuridine is not an inhibitor or a substrate of major human drug transporters.

Cedazuridine is an inhibitor of cytidine deaminase (CDA) and as such, drugs known to be metabolized by CDA should be avoided during treatment with ASTX727.

Concomitant proton pump inhibitor medication usage was shown not to have a significant impact on decitabine, cedazuridine, and cedazuridine-epimer parameters and subsequent exposures, based on population PK analysis.

As plasma protein binding of decitabine is negligible (<1%) and for cedazuridine is low (~34% to 38%), interactions due to displacement of more highly protein bound drugs from plasma proteins are not expected.

6.3.2 Venetoclax

Venetoclax is predominantly metabolized by CYP3A4 *in vitro*, thus CYP3A4 inhibitors or inducers are expected to cause changes in venetoclax exposures. Clinical studies have supported the *in vitro* observations for venetoclax as a sensitive substrate of CYP3A4: >5-fold increase in AUC when co-dosed with ketoconazole, and 71% decrease in AUC when co-dosed with rifampin. Venetoclax is a substrate for the efflux transporters P-gp and BCRP, and inhibitors or inducers of these transporters are expected to cause change in the exposure of venetoclax. Venetoclax is a P-gp and BCRP inhibitor and may interact with substrates for these transporters. Venetoclax may inhibit OATP1B1 and cause weak interaction with drugs that are substrates of this transporter. Refer to the venetoclax label for dose adjustment concerning concurrent medications.

6.3.2.1 Excluded Concomitant Medications and Procedures

Concomitant use of a strong CYP3A **inducer** should be avoided during study. Alternative treatments with less CYP3A induction should be considered. Strong or moderate CYP3A **inhibitors** may be necessary later in the study, but should be avoided D1-14.

Concomitant use of venetoclax with strong inhibitors (e.g., ketoconazole, itraconazole, posaconazole, voriconazole, ritonavir, clarithromycin) or moderate (e.g., erythromycin, ciprofloxacin, diltiazem, fluconazole, verapamil) inhibitors of CYP3A for CYCLE 1 D1-D14 is contraindicated.

- In the event that an investigator feels that a moderate or strong CYP3A4 inhibitor or inducer is necessary for the care of the patient in D1-14, the investigator will contact the PI to discuss and consider replacement of the patient for the purposes of PK/PD (and DLT for P1b) analyses.
- **After C1D14,** prophylactic antifungal therapy with strong or moderate inhibitors of CYP3A is permitted as per institutional guidelines if given consistent with the venetoclax dose modification guidelines below:
    - **If a strong CYP3A inhibitor must be used, reduce the dose of venetoclax to 70-100mg (see table).** Resume the dose that was used prior to initiating the CYP3A inhibitor or P-gp inhibitor 2 to 3 days after discontinuation of the inhibitor.
    - **If a moderate CYP3A inhibitor or a P-gp inhibitor must be used, reduce the dose of venetoclax by 50%.** Resume the dose that was used prior to initiating the CYP3A inhibitor or P-gp inhibitor 2 to 3 days after discontinuation of the inhibitor. If a narrow therapeutic index P-gp substrate must be used, it should be taken at least 6 hours before venetoclax.

• Coadministered Drug	• Ramp up Venetoclax Dose	• Steady Venetoclax Daily Dose
• Posaconazole	• Day 1: 10 mg • Day 2: 20 mg • Day 3: 50 mg • Day 4: 70 mg	• 70mg
• Other Strong CYP3A inh	• Day 1: 10 mg • Day 2: 20 mg • Day 3: 50 mg • Day 4: 100 mg	• 100mg
• Moderate CYP3A inh	• Reduce dose by 50%	
• P-gp inhibitor		

- Venetoclax should be administered using caution with substrates or inhibitors of BCRP or OATP1B1/B3.
- Venetoclax may cause a significant increase in C_{max} and AUC of warfarin. If venetoclax is co-administered with warfarin, the international normalized ratio (INR) should be monitored closely.
- Live attenuated vaccines should not be administered prior to, during, or after treatment with venetoclax until B-cell recovery occurs. The safety and efficacy of immunization with live attenuated vaccines during or following venetoclax therapy have not been studied. Patients should be advised that vaccinations may be less effective.
- Grapefruit products, Seville oranges, and starfruit during treatment should be avoided.

- Concomitant use of venetoclax with strong or moderate CYP3A inducers should be avoided during the study.

6.3.3 Daunorubicin and Cytarabine

Daunorubicin has Y-site intravenous incompatibility with cefepime hydrochloride, dexamethasone sodium phosphate, furosemide, heparin sodium, levofloxacin, pantoprazole, Piperacillin sodium-tazobactam sodium, and Sulfamethoxazole- trimethoprim among others. Daunorubicin has admixture intravenous incompatibility with dexamethasone sodium phosphate and heparin sodium. Cytarabine has Y-site intravenous incompatibility with allopurinol sodium, amiodarione, amphotericin B conventional colloidal, and daptomycin among others. Cytarabine has admixture intravenous incompatibility with heparin sodium, and regular insulin among others.

6.4 Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment with venetoclax + ASTX727 may continue for up to one year (12 cycles) or until one of the following criteria applies. Treatment with 7+3 chemotherapy (cytarabine + daunorubicin) may continue for up two induction cycles or until one of the following criteria applies.

- Disease progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Patient decides to withdraw from the study
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Clinical progression
- Patient non-compliance
- Pregnancy
    - All women of child bearing potential should be instructed to contact the investigator immediately if they suspect they might be pregnant (*e.g.*, missed or late menstrual period) at any time during study participation.
    - The investigator must immediately notify CTEP in the event of a confirmed pregnancy in a patient participating in the study.
- Termination of the study by sponsor
- The drug manufacturer can no longer provide the study agent

After completion of assigned therapy, patients in the control arm should receive standard post-remission consolidation with cytotoxic chemotherapy (per institutional standard practice, and allowing for accepted dose reductions from high dose cytarabine to as low as $1 g/m^2$ for patients 55-65 years old) and/or hematopoietic stem cell transplantation (HSCT). Randomization to the venetoclax/ASTX727 arm does not preclude referral to HSCT, but venetoclax/ASTX727 will continue until decision to proceed to HSCT is made as long as patient continues to derive benefit without significant toxicity.

HSCT is allowed for patients in remission, and patients proceeding to alloSCT will be followed for EFS but not for toxicity once preparative regimen for HSCT has begun. If the patient does not qualify, or knowingly denies consent for alloHSCT, despite knowing this is standard of care for eligible, higher risk AML patients, patients will be allowed to continue on study drug.

The reason(s) for protocol therapy discontinuation, the reason(s) for study removal, and the corresponding dates must be documented in the Case Report Form (CRF).

6.5 Duration of Follow-Up

Patients will be followed for three months after removal from study or until failure to achieve complete response or complete response with incomplete count recovery (CR/CRi), progression, or death, whichever occurs first. Patients removed from study for unacceptable AE(s) will be followed until resolution or stabilization of the AE(s). A single interim analysis for futility will be conducted when 32 patients experience an event (failure to achieve CR/CRi in 3 months, progression, or death) in the study. Patients will be followed for survival approximately every 3 months for up to 2 years via clinic visit, chart review, study team investigation or similar observation (e.g. Social Security death index), or by telephone.

7. DOSING DELAYS/DOSE MODIFICATIONS

7.1 CTEP Agents

7.1.1 Supportive Care Recommendations for ASTX727-Related Adverse Events

Supportive measures for optimal medical care should be provided to all patients in this study. In addition to the required prophylactic therapy with 5-HT3 antagonists, supportive care per institutional guidelines and/or the National Comprehensive Cancer Network® (NCCN) Clinical Practice Guidelines in Oncology (NCCN Guidelines®) should be used as clinically indicated at the discretion of the Investigator.

Supportive care guidelines for managing AEs are provided in the tables in the following sections.

7.1.2 Venetoclax Supportive Care and Dose Adjustment Guidelines

Toxicity and Intensity	Occurrence	Venetoclax Dose Modification
Tumor Lysis Syndrome		
Blood chemistry changes or symptoms suggestive of TLS	Any	• Withhold the next day's dose. If resolved within 24 to 48 hours of last dose, resume at the same dose. • For any blood chemistry changes requiring more than 48 hours to resolve, resume at a 50% dose reduction. • For any events of clinical TLS,[a] resume at a 50% dose reduction following resolution.
Non-Hematologic Toxicities		
Grade 3 or 4 non-hematologic toxicities	1st occurrence	• Interrupt venetoclax. Once the toxicity has resolved to Grade 1 or baseline level, venetoclax therapy may be resumed at the same dose. No dose modification is required.
	2nd and subsequent occurrences	• Interrupt venetoclax. Resume at 50% dose reduction following resolution. A larger reduction may occur at the discretion of the study physician.
Consider discontinuing venetoclax for patients who require dose reductions to <400 mg for more than 2 weeks. [a] Clinical TLS defined as laboratory TLS with clinical consequences such as acute renal failure, cardiac arrhythmias, or sudden death and/or seizures.		

7.1.3 Venetoclax and ASTX727 Dose Adjustments Guidelines for Cytopenias

ASTX727 and venetoclax each have potential to lead to hematologic toxicity, and AML patients have malfunctioning bone marrow as an element of their disease. In the first cycle of therapy, all doses of ASTX727 and venetoclax should be given, and doses should not be held for cytopenias, if possible. However, dose interruptions and dose reductions may occur during any cycle at the discretion of the investigator for safety. Guidelines for dose modifications for hematologic toxicities are noted below:

Recommended Dose Modifications for Hematologic Toxicities*

Event	Occurrence	Action
	Occurrence prior to achieving remission	• Transfuse blood products, administer prophylactic and treatment anti-infectives as clinically indicated. *(*see protocol section 6.3.2)*

Grade 4 Neutropenia with or without fever or infection *OR* **Grade 4 Thrombocytopenia**		• In most instances, venetoclax and ASTX727 cycles should not be interrupted due to cytopenias prior to achieving remission.
	First occurrence after achieving remission and lasting at least 7 days	• Delay subsequent treatment cycle of venetoclax and ASTX727 and monitor blood counts. • Administer G-CSF if clinically indicated for neutropenia. Once the toxicity has resolved to Grade 1 or 2, resume venetoclax therapy at the same dose in combination with ASTX727.
	Second occurrence in cycles after achieving remission and lasting 7 days or longer	• Delay subsequent treatment cycle of venetoclax and ASTX727 and monitor blood counts. • Administer G-CSF if clinically indicated for neutropenia. Once the toxicity has resolved to Grade 1 or 2, resume venetoclax therapy at same dose and the duration reduced by 7 days for each subsequent cycle with ASTX727 at same dose.
	Subsequent occurrences in cycles after achieving remission and lasting 7 days or longer	• Delay subsequent treatment cycle of venetoclax and ASTX727 and monitor blood counts. • Administer G-CSF if clinically indicated for neutropenia. Once the toxicity has resolved to Grade 1 or 2, resume venetoclax therapy at same dose and the duration reduced by 7 days for each subsequent cycle with ASTX727 therapy at D1-4 days for each subsequent cycle.

* This table was modified from the current Venclexta USPI.

Further dose reductions and cycle length adjustments should be discussed with the PI.

7.1.4 ASTX727/Venetoclax Dose Adjustment in the Setting of Infection

Appropriate broad-spectrum IV antibiotics and antiviral agents should be started immediately in patients who develop neutropenic fever or other signs of systemic infection. If fungal infection is suspected, antifungal agents may be used, with appropriate dose reductions in venetoclax if antifungal therapy includes a contraindicated medicine (See section 8.1.1). If patient is beyond

C1D19 (*e.g.* 2 weeks after completion of induction), antifungal secondary prophylaxis, with appropriate dose reductions of venetoclax, may be employed by the treating physician.

If patient has active AML (*e.g.* recent induction, circulating AML blasts), dosing decision is deferred to the treating physician as untreated AML is lethal, and neutropenic fever may be from AML. If the treating physician deems that the patient has greater opportunity for benefit with ongoing therapy with investigational agent than not, continued administration of therapy is recommended. If patient has neutropenic fever without evidence of active AML, hold venetoclax dosing and delay next cycle of ASTX727 and venetoclax until patient's condition is stable, and ANC is grade </=2. Resume dose at previous dose level.

If the neutropenic fever is persistent, or for ≥ 2 occurrences, recommend consulting with the principal investigator.

Consider institution of colony stimulating factors at investigator's discretion/institutional guidelines.

Missed doses will not be replaced. Patients may continue on antibiotics for prolonged periods while re-initiating their treatment at the discretion of the Investigator.

7.1.5 Conditions Not Requiring ASTX727/Venetoclax Dose Reduction

The following conditions are exceptions to the dose-modification guidelines. ASTX727 does not need to be held in the following cases:

- Alopecia of any grade.
- Electrolyte or serum analyte (*e.g.,* urate) abnormalities that are reversible with standard interventions.

Isolated values of ≥Grade 3 alkaline phosphatase. Determination of liver versus bone etiology should be made, and evaluation of gamma-glutamyl transferase, 5' nucleotidase, or other liver enzymes should be performed.

7.2 Commercial Agents

Below are proposed guidelines for adjustments, but investigators should determine the most appropriate course of action based on the specifics of the situation and circumstances for each individual patient. Patients should be weighed and BSA should be calculated prior to CxD1 dosing. A BSA change of >10% will necessitate a dose adjustment in cytarabine/daunorubicin arm.

7.2.1 Cytarabine

Hepatic Impairment
Dosage should be modified depending on clinical response and degree of hepatic impairment, but no quantitative recommendations are available.

Renal Impairment

CrCl >60 mL/min: no dosage adjustment needed.
CrCl ≤60 mL/min: increased risk of cerebellar and cerebral toxicity with higher dose regimens (only applicable in consolidation rounds). A recommended dosage adjustment is as follows: for a serum creatinine of 1.5–1.9 mg/dL or increase of serum creatinine from baseline of 0.5–1.2 mg/dL during treatment, reduce the dose of cytarabine to 1 g/m^2/dose. for a serum creatinine ≥2 mg/dL or if the change in baseline serum creatinine was >1.2 mg/dL, hold the dose of cytarabine and discuss with study chair.

7.2.2 Daunorubicin

Hepatic Impairment
Total bilirubin 1.2–3 mg/dL: **REDUCE RECOMMENDED DOSE BY 50%.**
Total bilirubin >3–5 mg/dL: reduce recommended dose by 75%.
Total bilirubin >5 mg/dL: omit dose.

Renal Impairment
It is recommended in patients with a serum creatinine >3 mg/dL to administer 50% of the dose.

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational or commercial agents administered in this study can be found in Section 10.1.

8.1 CTEP IND Agents

8.1.1 Venetoclax (NSC 766270)

Chemical Name: 4-(4-{[2-(4-chlorophenyl)-4,4- dimethylcyclohex-1-en-1-yl]methyl}piperazin-1-yl)-N-({3-nitro-4-[(tetrahydro-2H-pyran-4-ylmethyl)amino]phenyl}sulfonyl)-2-(1H-pyrrolo[2,3-b]pyridin-5-yloxy)benzamide)

Other Names: ABT-199, A-1195425.0, Venclexta™, Venclyxto™, GDC-0199, RO5537382

CAS registry number: 1257044-40-8

Molecular Formula: $C_{45}H_{50}ClN_7O_7S$ **M.W.:** 868.44 g/mol

Approximate Solubility: very low aqueous solubility

Mode of Action: B-cell lymphoma-2 (BCL2) inhibitor

Description:
Genetech/AbbVie supplies and CTEP, DCTD, NCI distributes Venetoclax tablets as oblong, bi-convex pale yellow or beige tablets that contain 10 mg, 50 mg or 100 mg venetoclax as the active ingredient. Each tablet also contains the following inactive

ingredients: copovidone, colloidal silicon dioxide, polysorbate 80, sodium stearyl fumarate, and calcium phosphate dibasic. In addition, the 100 mg coated tablets include the following: iron oxide yellow, polyvinyl alcohol, polyethylene glycol, talc, and titanium dioxide. Each tablet is debossed with "V" on one side and a "10", "50" or "100" corresponding to the tablet strength on the other side. The 10 mg and 50 mg supplies will be provided in blister cards. Each 10 mg blister card will contain 16 tablets, while each 50 mg blister card will contain 8 tablets. The 100 mg tablets will be supplied in 32 count bottles.

How Supplied: 10 mg, 50 mg, 100 mg tablets

Storage: Store between 15° to 25°C (59° to 77°F)

If a storage temperature excursion is identified, promptly return venetoclax to between 15° to 25°C or below and quarantine the supplies. Provide a detailed report of the excursion (including documentation of temperature monitoring and duration of the excursion) to PMBAfterHours@mail.nih.gov for determination of suitability.

Stability: Stability studies are ongoing.

Route(s) **of Administration:** oral

Method of Administration:

Tablets should be taken orally once daily, at approximately the same time, with a meal and water. Do not chew, crush, or break tablets.

If a dose is missed by less than 8 hours, take the missed dose right away and take the next dose as usual. If a dose is missed by more than 8 hours, the patient should wait and take the next dose at the usual time.

Patients should not take an additional dose if they vomit after taking venetoclax. They should take the next dose at the usual time the following day.

Potential Drug Interactions:

Venetoclax is predominantly metabolized by CYP3A4/5. Concomitant use of venetoclax with strong inhibitors of CYP3A at initiation (D1-D14 of cycle 1) and during ramp-up phase is contraindicated. For patients who have completed the ramp-up phase and D1-14 of cycle 1 and are on a steady daily dose, reduce the dose by at least 75% when used concomitantly with strong CYP3A inhibitors. Resume the venetoclax dose that was used prior to initiating the CYP3A inhibitor 2 to 3 days after discontinuation of the inhibitor.

Avoid concomitant use of moderate CYP3A inhibitors or P-gp inhibitors. If a moderate CYP3A inhibitor or a P-gp inhibitor must be used, reduce the dose by at least 50%. Resume the dose that was used prior to initiating the CYP3A inhibitor or P-gp inhibitor 2 to 3 days after discontinuation of the inhibitor. If a narrow therapeutic index P-gp substrate must be used, it should be taken at least 6 hours before venetoclax.

Avoid concomitant use with strong CYP3A inducers or moderate CYP3A inducers.

Venetoclax may cause a significant increase in Cmax and AUC of warfarin. International normalized ratio (INR) should be monitored closely in patients receiving warfarin.

Venetoclax had no large effect on QTc interval (i.e., > 20 ms) and there was no relationship between venetoclax exposure and change in QTc interval.

Gastric acid reducing agents (e.g., proton pump inhibitors, H2-receptor antagonists, antacids) do not affect venetoclax bioavailability.

Avoid grapefruit products, Seville oranges, and starfruit during treatment

Patient Care Implications:

Tumor Lysis Syndrome (TLS) prophylaxis - Patients should be instructed to drink 6 to 8 glasses water each day, starting 2 days before the first dose, on the day of the first dose of venetoclax, and each time the dose is increased. Consider IV hydration and/or uric acid reducing agents in patients at risk for TLS.

Immunization: Do not administer live attenuated vaccines prior to, during, or after venetoclax treatment until B-cell recovery has occurred.

Females of reproductive potential should use effective contraception during treatment and for at least 30 days after the last dose.

Nursing women should discontinue breastfeeding during treatment with venetoclax.

Availability

Venetoclax is an investigational agent supplied to investigators by the Division of Cancer Treatment and Diagnosis (DCTD), NCI.

Venetoclax is provided to the NCI under a Collaborative Agreement between the Pharmaceutical Collaborator and the DCTD, NCI).

8.1.2 ASTX727 (NSC 820631)

Chemical Name (Cedazuridine):
(4R)-1-[(2R,4R,5R)-3,3-difluoro-4-hydroxy-5- (hydroxymethyl)oxolan-2-yl]-4-hydroxy-1,3-diazinan-2-one
Chemical Name (Decitabine):
4-amino-1-[(2R,4S,5R)-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl]-1,3,5-triazin-2-(1H)-one

Other Names: Cedazuridine and decitabine; INQOVI®

Classification: Cytidine deaminase inhibitor and DNA hypomethylating agent

CAS Registry Number (Cedazuridine): 1141397-80-9

CAS Registry Number (Decitabine): 2353-33-5

Molecular Formula (Cedazuridine): C9H14F2N2O5 M.W.: 268.21 Daltons

Molecular Formula (Decitabine): C8H12N4O4 M.W.: 228.21 Daltons

Approximate Solubility of Cedazuridine: Soluble in dimethylsulfoxide, sparingly soluble in water and 50 mM phosphate, slightly soluble in methanol, and insoluble in acetone, and acetonitrile. Not hygroscopic.

Approximate Solubility of Decitabine: Freely soluble in dimethyl sulfoxide, slightly soluble in water, very slightly soluble in methanol. Hygroscopic.

Mode of Action: Decitabine is a cytidine nucleoside analog that inhibits DNA methylation, thereby causing hypomethylation of DNA and cellular differentiation and/or apoptosis. Cytidine deaminase (CDA) is an enzyme that catalyzes the degradation of cytidine, including the cytidine analog decitabine. High levels of CDA in the gastrointestinal tract and liver degrade decitabine and limit its oral bioavailability. Cedazuridine is a CDA inhibitor. Administration of cedazuridine with decitabine increases systemic exposure of decitabine.

How Supplied: Astex Pharmaceuticals Inc. supplies and PMB distributes, ASTX727 as a red, film-coated, oval, immediate-release, fixed dose combination (FDC) tablet containing the cedazuridine (100 mg) and decitabine (35 mg).

Inactive ingredients consist of lactose, hypromellose, croscarmellose sodium, colloidal silicon dioxide, magnesium stearate, and Opadry II 85F15458 Red. The Opadry is a tablet coating formula that contains titanium dioxide, polyvinyl alcohol, polyethylene glycol, talc, and iron oxide red. Tablets are plain faced on both sides. Future clinical supply may contain a debossed marking of "H35" on each tablet.

ASTX727 is packaged as 5 tablets per bottle. The bottles are white opaque high-density polyethylene (HDPE) with a child resistant closure containing 1 or 2 desiccant canisters for moisture absorption. Bottles have an induction seal.

Storage: Store intact/unopen bottles at 20°C to 25°C (68°F to 77°F); excursions permitted to 15°C to 30°C (59°F to 86°F).

If a storage temperature excursion is identified outside of the permitted excursion 15°C to 30°C (59°F to 86°F), promptly return ASTX727 to 20°C to 25°C (68°F to 77°F) and quarantine the supplies. Provide a detailed report of the excursion (including documentation of temperature monitoring and duration of the excursion) to PMBAfterHours@mail.nih.gov for determination of

suitability.

Route and Method of Administration: Oral. Take ASTX727 tablet approximately at the same time on an empty stomach. Do not eat, drink milk or alcohol 2 hours before and 2 hours after taking ASTX727. Clear liquids such as water, black coffee, or tea are allowed. Take whole tablets with 8 oz. (240 mL) of water; do not crush, cut, or chew the tablet.

Missed dose within 12 hours of the scheduled time can be made up. Patient needs to take as soon as possible and resume the next day on schedule. If the patient fails to make up the dose within 12 hours, the dose should be resumed at the scheduled time the next day.

Potential Drug Interactions: Decitabine had no clinically meaningful effect on the pharmacokinetics of cedazuridine. Cedazuridine increased the exposure of decitabine.

Cedazuridine is an inhibitor of cytidine deaminase (CDA) and as such, drugs known to be metabolized by CDA should be avoided during treatment with ASTX727.

CYP450: Cedazuridine is not a substrate of cytochrome P450 (CYP) enzymes. Cedazuridine does not induce CYP1A, CYP2B6, CYP2C9, or CYP3A or inhibit CYP1A, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A.

Protein Transporters: Cedazuridine is not a substrate of P-glycoprotein (P-gp), MATE1, MATE2-K, OAT1, OAT3, OATP1B1, OATP1B3, OATP2B1, OCT1, or OCT2, and does not inhibit P-gp, BCRP, MATE1, MATE2-K, OAT1, OAT3, OATP1B1, OATP1B3, or OCT2.

Concomitant use of proton pump inhibitor does not have significant impact on decitabine or cedazuridine exposure.

Protein binding: Plasma protein binding of decitabine is negligible (<1%) and for cedazuridine is low (~34% to 38%), therefore interactions due to displacement of more highly protein bound drugs from plasma proteins are not expected.

Patient Care: ASTX727 can cause fetal harm when administered to pregnant women. For that reason, women of childbearing potential must use highly effective contraception during treatment with ASTX727 and for at least 6 months after the last dose. Men with female partners of childbearing potential should be advised to practice highly effective contraceptive measures of birth control and not to father a child while receiving treatment with decitabine and for 3 months after the last dose. Breastfeeding is not allowed during the study.

Availability

ASTX727 is an investigational agent supplied to investigators by the Division of Cancer Treatment and Diagnosis (DCTD), NCI.

ASTX727 is provided to the NCI under a Collaborative Agreement between the Pharmaceutical Collaborator and the DCTD, NCI (see Section 13.5).

8.1.3 Agent Ordering and Agent Accountability

8.1.3.1 NCI-supplied agents may be requested by eligible participating Investigators (or their authorized designee) at each participating institution. The CTEP-assigned protocol number must be used for ordering all CTEP-supplied investigational agents. The eligible participating investigators at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA Form 1572 (Statement of Investigator), NCI Biosketch, Agent Shipment Form, and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP-supplied investigational agents for the study should be ordered under the name of one lead participating investigator at that institution.

Confirmation of patient enrollment is required for initial drug shipment.

Submit agent requests through the PMB Online Agent Order Processing (OAOP) application. Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account and the maintenance of an "active" account status, a "current" password, and active person registration status. For questions about drug orders, transfers, returns, or accountability, call or email PMB any time. Refer to the PMB's website for specific policies and guidelines related to agent management.

8.1.3.2 Agent Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a careful record of the receipt, dispensing and final disposition of all agents received from the PMB using the appropriate NCI Investigational Agent (Drug) Accountability Record (DARF) available on the CTEP forms page. Store and maintain separate NCI Investigational Agent Accountability Records for each agent, strength, formulation and ordering investigator on this protocol.

8.1.4 Investigator Brochure Availability

The current versions of the IBs for the agents will be accessible to site investigators and research staff through the PMB OAOP application. Access to OAOP requires the establishment of a CTEP IAM account and the maintenance of an "active" account status, a "current" password and active person registration status. Questions about IB access may be directed to the PMB IB Coordinator via email.

8.1.5 Useful Links and Contacts

- CTEP Forms, Templates, Documents: http://ctep.cancer.gov/forms/
- NCI CTEP Investigator Registration: RCRHelpDesk@nih.gov
- PMB policies and guidelines: http://ctep.cancer.gov/branches/pmb/agent_management.htm
- PMB Online Agent Order Processing (OAOP) application: https://ctepcore.nci.nih.gov/OAOP

- CTEP Identity and Access Management (IAM) account: https://ctepcore.nci.nih.gov/iam/
- CTEP IAM account help: ctepreghelp@ctep.nci.nih.gov
- IB Coordinator: IBCoordinator@mail.nih.gov
- PMB email: PMBAfterHours@mail.nih.gov
- PMB phone and hours of service: (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET)

8.2 Commercial Agents

8.2.1 Cytarabine (NSC 63878)

Other Names: ara-C

Chemical Name: 4-amino-1-β-Darabinofuranosyl-2(1H)-pyrimidinone

Classification: antineoplastic

Molecular Weight: 243.217 g/mol

CAS Number: 63878

Mode of Action: Cytarabine inhibits DNA synthesis. Cytarabine gains entry into cells by a carrier process, and then must be converted to its active compound, aracytidine triphosphate. Cytarabine is a pyrimidine analog and is incorporated into DNA; however, the primary action is inhibition of DNA polymerase resulting in decreased DNA synthesis and repair. The degree of cytotoxicity correlates linearly with incorporation into DNA; therefore, incorporation into the DNA is responsible for drug activity and toxicity. Cytarabine is specific for the S phase of the cell cycle (blocks progression from the G1 to the S phase).

Side Effects: Refer to drug package insert for additional information.

Side effects include:

- Cardiovascular: Angina pectoris, chest pain, local thrombophlebitis, pericarditis
- Central nervous system: Aseptic meningitis, cerebral dysfunction, dizziness, headache, neuritis, neurotoxicity, paralysis (intrathecal and IV combination therapy), reversible posterior leukoencephalopathy syndrome
- Dermatologic: Acute generalized exanthematous pustulosis, alopecia, dermal ulcer, ephelis, pruritus, skin rash, urticaria
- Endocrine & metabolic: Hyperuricemia
- Gastrointestinal: Abdominal pain, anal fissure, anorexia, diarrhea, esophageal ulcer, esophagitis, increased serum amylase, increased serum lipase, intestinal necrosis, mucositis, nausea, pancreatitis, sore throat, toxic megacolon, vomiting
- Genitourinary: Urinary retention
- Hematologic & oncologic: Anemia, bone marrow depression, hemorrhage, leukopenia, megaloblastic anemia, neutropenia (onset: 1 to 7 days; nadir [biphasic]: 7 to 9 days and

at 15 to 24 days; recovery [biphasic]: 9 to 12 days and at 24 to 34 days), reticulocytopenia, thrombocytopenia (onset: 5 days; nadir: 12 to 15 days; recovery 15 to 25 days)
- Hepatic: Hepatic insufficiency, hepatic sinusoidal obstruction syndrome (formerly known as hepatic veno-occlusive disease), increased serum transaminases (acute), jaundice
- Hypersensitivity: Allergic edema, anaphylaxis
- Infection: Sepsis
- Local: Cellulitis at injection site, inflammation at injection site (SC injection), local inflammation (anus), pain at injection site (SC injection)
- Neuromuscular & skeletal: Rhabdomyolysis
- Ophthalmic: Conjunctivitis
- Renal: Renal insufficiency
- Respiratory: Acute respiratory distress, dyspnea, interstitial pneumonitis
- Miscellaneous: Drug toxicity (cytarabine syndrome; chest pain, conjunctivitis, fever, maculopapular rash, malaise, myalgia, ostealgia), fever

Availability: Commercially available.

Product Description: Cytarabine is an odorless, white to off-white, crystalline powder which is freely soluble in water and slightly soluble in alcohol and in chloroform. Cytarabine is commercially available.

Preparation: Clear colorless solution. Refer to standard local procedure or the package insert for standard preparation instructions.

Storage and Stability: From a microbiological point of view, the product should be used immediately. If not used immediately, in-use storage times and conditions are the responsibility of the user and would normally not be longer than 24 hours at 2-8°C, unless dilution has taken place in controlled and validated aseptic conditions.

Route of Administration: Continuous IV, per institutional guidelines.

Additional information can be found in the package insert for cytarabine.

8.2.2 Daunorubicin (NSC 82151)

Other Names: Cerubidin

Classification: antineoplastic

Molecular Weight: 527.52 g/mol

CAS Number: 82151

Mode of Action: Daunorubicin inhibits DNA and RNA synthesis by intercalation between

DNA base pairs and by steric obstruction. Daunomycin intercalates at points of local uncoiling of the double helix. Although the exact mechanism is unclear, it appears that direct binding to DNA (intercalation) and inhibition of DNA repair (topoisomerase II inhibition) result in blockade of DNA and RNA synthesis and fragmentation of DNA.

Side Effects: Refer to drug package insert for additional information.

Side effects include:

- >10%:
    - Cardiovascular: Cardiac failure (dose-related, may be delayed for 7 to 8 years after treatment), ECG abnormality (transient, generally asymptomatic and self-limiting; includes atrial premature contractions, ST segment changes on ECG, supraventricular tachycardia, ventricular premature contractions)
    - Dermatologic: Alopecia (reversible)
    - Gastrointestinal: Nausea (mild), stomatitis, vomiting (mild)
    - Genitourinary: Red urine discoloration
    - Hematologic & oncologic: Bone marrow depression (onset: 7 days; nadir: 10 to 14 days; recovery: 21 to 28 days; primarily leukopenia; anemia, thrombocytopenia)
    - Miscellaneous: Radiation recall phenomenon
- 1% to 10%:
    - Dermatologic: Discoloration of sweat
    - Endocrine & metabolic: Hyperuricemia
    - Gastrointestinal: Abdominal pain, diarrhea, discoloration of saliva, gastrointestinal ulcer
    - Local: Post-injection flare
    - Ophthalmic: Discoloration of tears

Availability: Commercially available

Product Description: Daunorubicin hydrochloride is the hydrochloride salt of an anthracycline cytotoxic antibiotic produced by a strain of *Streptomyces coeruleorubidus*. It is provided as a deep red sterile liquid in vials for intravenous administration only. Its molecular formula is C27H29NO10•HCl with a molecular weight of 563.99. It is a hygroscopic crystalline powder. The pH of a 5 mg/mL aqueous solution is 4 to 5. Daunorubicin is commercially available.

Preparation: Deep red liquid. Refer to the package insert for standard preparation instructions.

Storage and Stability: Stored at 25°C (77°F); excursions permitted to 15°C–30°C (59°F–86°F). Needs to be protected from moisture.

Route of Administration: infused over 10 to 30 minutes. IV push route is not recommended, but may be used as per institutional standards.

Additional information can be found in the package insert for daunorubicin.

9. STATISTICAL CONSIDERATIONS

9.1 Study Design/Endpoints

This open-label randomized phase 2 clinical trial comparing response between groups of patients with $FLT3^{wt}$ poor risk AML who receive either venetoclax + ASTX727 (venetoclax+ASTX727) or standard anthracycline induction therapy ('7+3'). Responses designated as CR include: Bone marrow blasts <5%; absence of blasts with Auer rods; absence of extramedullary disease; absolute neutrophil count >1.0 x 10^9/L (1000/L); platelet count >100 x10^9/L (100 000/L); independence of red cell transfusions. Complete remission with incomplete blood count recovery (CRi) consists of a marrow CR with failure to reach ANC or platelet count recovery as noted with CR. Primary analysis will be conducted as Intent to Treat (ITT) and performed using all randomizable patients. Patients lost to follow-up will be censored. Based on our own previous clinical trial data, the median EFS in this group of AML patients was 3.9 months. Assuming 24 months of accrual, 4 months of follow-up and a maximum 2% per month loss to follow-up rate, 55 patients (2.3 patients per month) provide 81% power to detect (one-sided $p<0.1$) a true hazard ratio of 2.0 (increase in median survival from 3.9 months to 7.8 months). The prior information on efficacy in the VEN+ASTX727 patient population is limited. Consequently, we will apply a futility stopping rule when 50% of the 40 expected events (E=20) have been observed. This will stop the trial for futility when 50% of expected events have occurred if the observed hazard ratio at that time favors the 7+3 arm (Wieand *et al*., 1994). Primary analysis will occur once 40 EFS events have been observed.

General Analysis Considerations

In the clinical trial, EFS will be documented along with response to therapy (CR, CRi, PR, SD and progression), duration of response, progression-free survival and overall survival. Statistical analysis of EFS will begin when 40 EFS events are observed. For the monitoring of safety, adverse events will be graded according to CTCAE v 5.0. All patients will be followed for as long as 2 years for disease progression or death. Patients alive and progression-free at the time of last follow-up will be censored. Overall survival (OS) is defined as the time from randomization to death for any reason. Patients alive at last follow-up will be censored.

Categorical data (*e.g.* response, adverse events) will be summarized in frequency tables with 95% Wilson confidence intervals as their measures of precision. Continuous variables (*e.g*. age, lab values) will be summarized using the minimum and maximum values along with the 25th, 50th (median) and 75th percentiles. If normality is reasonable, the mean and standard deviation will also be estimated. 95% confidence intervals will be estimated for the median using the bootstrap and via normal theory for mean. The distributions of PFS and OS will be estimated using the method of Kaplan and Meier. Hazard rates for PFS and OS will be estimated, along with 95% confidence intervals, using the Cox (proportional hazards) regression model.

9.2 Sample Size/Accrual Rate

The hypothesis EFS among patients treated with venetoclax+ASTX727 is superior to the EFS among patients treated with standard anthracycline induction therapy ('7+3') will be tested. Based on our own previous clinical trial data, the median EFS for this group of AML patients was 3.9 months. Assuming 24 months of accrual, 4 months of follow-up and a maximum 2% per month loss to follow-up rate, 55 patients (2.3 patients per month) provide 81% power to detect (one-sided $p<0.1$) a true hazard ratio of 2.0 (increase in median event free survival from 3.9 months to 7.8 months).

PLANNED ENROLLMENT REPORT

DOMESTIC PLANNED ENROLLMENT REPORT (TREATMENT)					
Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	0	0	0	0	0
Asian	3	0	0	0	3
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Black or African American	4	4	2	0	10
White	19	18	1	1	38
More Than One Race	2	2	0	0	4
Total	27	24	3	1	55

PHS 398 / PHS 2590 (Rev. 03/20 Approved Through 2/28/2023) OMB No. 0925-0001/0002

9.3 Analysis of Secondary Endpoints

For the Phase 2 portion the following endpoints will be analyzed:

- The complete response (CR + CRi) rate in patients with treatment naïve AML treated with venetoclax and ASTX727.
- The duration of response (DoR) in patients with treatment naïve AML treated with venetoclax and ASTX727. DOR is defined as time from the date of first objective response until disease progression or death for any reason.
- The progression free survival (PFS) of patients with treatment naïve AML treated with venetoclax and ASTX727. PFS is defined as the time from randomization date to the date of progression of AML or death for any reason. Patients alive and progression-free at last follow-up will be censored.
- The overall response rate (ORR) (PR+CRi+CR) in patients with treatment naïve AML treated with venetoclax and ASTX727.
- The overall survival (OS) of patients with treatment naïve AML treated with venetoclax

and ASTX727. OS is defined as the time from randomization to death for any reason. Patients alive at last follow-up will be censored. Every patient will be followed for a minimum of 2 years. Patients not experiencing an event by 2 years will administratively censored.

- The proportion of treatment naïve AML patients receiving stem cell transplantation (SCT) following treatment with venetoclax and ASTX727.
- Mutational burdens in venetoclax+ASTX727 sensitive vs. resistant AML LICs.

DOR, PFS and OS are susceptible to the competing risk of bone marrow transplant, which will be coded as a competing risk if it occurs prior to progression or death as appropriate.

Secondary endpoints are focused on estimation; thus, no formal hypothesis testing will be performed. All point estimates of outcome measures will be accompanied by 95% (*e.g.* based on standard Gaussian methods or bootstrap methods, as appropriate) confidence intervals.

Continuous (*e.g.*, age at randomization) variables will be summarized using the 25th, 50th (median), and 75th percentiles, the range, as well as the mean and standard deviation. Categorical variables, (e.g., AE) will be reported as frequencies. 95% confidence intervals will be reported for all outcomes. Categorical variables will be compared among important prognostic groups using the chi-square test, or Fisher's exact test, as appropriate. Similar comparisons of continuous variables will be made using the two-sample t-test, paired t-test, or their nonparametric analogs, as appropriate. The distributions of EFS, DOR, PFS, and OS will be estimated using the method of Kaplan and Meier and compared among treatments and other important prognostic groups using the logrank test. Multivariable prediction modelling will be conducted under the generalized linear model approach with link functions appropriate to the scale of the outcome (e.g. logit for logistic regression for CR/CRi). The Cox (proportional hazards) regression model for competing risks will be used to model multivariable effects on EFS, DOR, PFS, and OS endpoints (Fine *et al.*, 1999). Standard residual and goodness-of-fit analyses will be conducted.

Single-cell RNA-seq data will first go through reads preprocessing, deconvolving, mapping and quantification steps. In detail, reads with unexpected structure will be removed and reads with poor quality will be trimmed using Trimmomatic. Reads will be sorted by their origin and aligned to the reference transcriptome, and mapped reads will be quantified into UMI-filtered counts per gene. Cells with unusually high numbers of low-quality reads will be flagged for removal. Additionally, cells with low proportion of uniquely mapping reads, low proportion of expressed genes (<500) or high proportion of reads mapped to the mitochondrial RNA (>10%) will be considered as low quality and excluded from downstream analysis. Systematic biases, batch effects or outlier samples will be detected using our own tool (https://github.com/liuqivandy/scRNABatchQC). After quality control, scRNA-seq data will be normalized using UMI-filtered counts. Technical and biological variance, including capture efficiency, sequencing efficiency, or cell-cycle stage, will be estimated and adjusted by the R package Seurat (Butler *et al.*, 2018). Batch effects if any will be corrected using ComBat or matching mutual nearest neighbors (Haghverdi *et al.*, 2018). Cell subpopulations will be identified by the shared nearest neighbor modularity optimization and visualized by UMAP using Seurat based on the first 50 principal components generated from the top 1000 highly

variable genes. Cell composition diversity between responders and non-responders will be compared using sc-UniFrac (https://github.com/liuqivandy/scUnifrac) (Fernandez *et al.*, 2009; Liu et al, 2018; Eisfeld *et al.*, 2018). Based on sc-UniFrac distance, a multidimensional scaling will be generated to provide a visual representation of the pattern of cell composition landscapes across patients. To identify changes in cell proportions between responders and nonresponders, we will use Mann-Whitney test and Dirichlet-multinomial regression that each capture distinct but complementary types of information. Mann-Whitney test will be performed to compare the proportions of each cell population in responders versus nonresponders. Additionally, Dirichlet-multinomial regression model will test for differences in cell composition between response states while accounting for the proportions of all of the other cell populations. Monocle 3 will be used to infer the pseudotemporal ordering of cells and to identify genes that are enriched along particular branches in the pseudotime tree (Trapnell et al., 2014). Differential expression in treatment versus pre-treatment will be performed using MAST, which fits a hurdle model to the expression of each gene, consisting of logistic regression for the drop out event and linear regression for the continuous expression level. The regression model will capture the effects of the cell population and the treatment state on gene expression, while controlling for cell complexity. The significant differential expression will require a minimum fold change of 1.5 and FDR of 0.01 and to be expressed by at least 1% of the cells within the group of interest.

We have used mutational analysis to obtain resistance signatures previously(Shastri *et al.*, 2018; Schinke *et al*., 2015) and will use these protocols in carrying forward with these analyses. The rate of mutations seen on deep sequencing of AML approaches 100% of patients (Cancer Genome Atlas Research 2013). The quality of the raw sequencing data will be analyzed by FastQC v0.11.4 with genome alignment (hg19) using Bowtie2 v2.2.9. For detection of somatic mutations, we will use Mutect2 of GATK v3.7 comparing each of the cell populations to the matched germline control with the default parameters. High-confidence mutations consistently detected by both Mutect2 and FreeBayes will be used for downstream analysis. In addition, to address potential false-negatives due to tumor cell contamination of germline controls, we will also include somatic mutations reported in MDS or AML by more than two groups in the COSMIC database (http://cancer.sanger.ac.uk/cosmic). Thereafter, we will exclude the mutations that were: (i) covered less than 20x in germline control or test cells; (ii) supported by <3 reads or 5% of the reads in test samples; (iii) reported in dbSNP database (SNPs v147), 1000 genome phase 3, or ExAC database 1.0 with population frequency >0.5%. Clonal analysis: VAF for each mutation will be calculated by the number of reads supporting the variant divided by total reads, using the FreeBayes output. Moreover, sample purity and local copy number variation (CNV) will estimated by the FACETS v0.5.6 package of R v3.2.3, which utilizes the read counts of both heterozygous and homozygous single-nucleotide polymorphism (SNP) loci. The CCF of each mutation will be estimated using the VAF, purity, and local CNV of the mutation as described before. When comparing the clonal architecture of different cell populations of the responding and resistant patients, we will generate a combined list of mutations that are covered at least 20x in all samples, then subject the VAFs of mutations in different populations to SciClone analysis. Mutational burderns will be compared between responders and nonresponders using t-test or wilcox rank sum test. Fisher's exact test will be used to find mutations enriched in responders compared to nonresponders. Muations will be summarized at the gene level to detected significantly mutated genes as well.

Plasma venetoclax concentration-time profiles will be analyzed by population pharmacokinetic (PPK) modeling using the software program NONMEM. Pharmacokinetic parameters (Cmax, AUC, CL/F, V/F, and t1/2) will be estimated. Concurrent medications will be grouped as strong or moderate CYP3A4 inhibitors and/or transporter (P-gp, BCRP, OATP1B1) inhibitors and included as a covariate in the PPK model. Relationships between PK parameters such as Cmax and AUC with toxicity or response will be performed using standard t-tests or Wilcoxon rank sum tests and illustrated using boxplots or other graphical displays. Values will be log transformed as appropriate to reflect biologic plausibility

9.4 For phase 2 protocols only: Reporting and Exclusions

9.4.1 Evaluation of Toxicity

All patients will be evaluable for toxicity from the time of their first treatment with venetoclax + ASTX727.

9.4.2 Evaluation of Response

All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations. Each patient will be assigned one of the following categories: 1) complete response, 2) complete response with incomplete count recovery 3) complete response with partial recovery counts 4) partial response, 5) stable disease, 6) progressive disease, 7) early death from malignant disease, 8) early death from toxicity, 9) early death because of other cause, or 10) unknown (not assessable, insufficient data). [Note: By arbitrary convention, category 10 usually designates the "unknown" status of any type of data in a clinical database.]

All of the patients who met the eligibility criteria (with the possible exception of those who received no study medication) should be included in the main analysis of the response rate. Patients in response categories 4-10 should be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate.

All conclusions should be based on all eligible patients. Subanalyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (*e.g.*, early death due to other reasons, early discontinuation of treatment, major protocol violations, *etc.*). However, these subanalyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported.

9.5 Data Safety Monitoring Board

The conduct of the randomized phase 2 component of this study will be overseen by the ETCTN DSMB. The DSMB will be responsible for recommendations to the Principal Investigator and NCI regarding possible trial closure and/or early reporting of the study. The study team (with the exception of the study statistician) will not have access to the summary outcome data until

released by the DSMB.

10. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs (Section 10.1) and the characteristics of an observed AE (Sections 10.2 and 10.3) will determine whether the event requires expedited reporting via the CTEP Adverse Event Reporting System (CTEP-AERS) **in addition** to routine reporting.

10.1 Comprehensive Adverse Events and Potential Risks Lists (CAEPRs)

10.1.1 CAEPRs for CTEP IND Agents

10.1.1.1 CAEPR for venetoclax:

Comprehensive Adverse Events and Potential Risks list (CAEPR)
for
Venetoclax (ABT-199, NSC 766270)

The Comprehensive Adverse Events and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 1298 patients.* Below is the CAEPR for Venetoclax (ABT-199).

NOTE: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Version 2.1, May 8, 2019[1]

Adverse Events with Possible Relationship to Venetoclax (ABT-199) (CTCAE 5.0 Term) [n= 1298]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Anemia			***Anemia (Gr 2)***
	Febrile neutropenia		
GASTROINTESTINAL DISORDERS			
	Constipation		***Constipation (Gr 2)***
Diarrhea			***Diarrhea (Gr 2)***
Nausea			***Nausea (Gr 2)***
	Vomiting		***Vomiting (Gr 2)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
Fatigue			***Fatigue (Gr 2)***

Adverse Events with Possible Relationship to Venetoclax (ABT-199) (CTCAE 5.0 Term) [n= 1298]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Fever		***Fever (Gr 2)***
INFECTIONS AND INFESTATIONS			
Infection[2]			***Infection[2] (Gr 2)***
INVESTIGATIONS			
	Lymphocyte count decreased		
Neutrophil count decreased			***Neutrophil count decreased (Gr 2)***
	Platelet count decreased		***Platelet count decreased (Gr 2)***
	White blood cell decreased		
METABOLISM AND NUTRITION DISORDERS			
	Hypocalcemia		
	Hypokalemia		***Hypokalemia (Gr 2)***
	Hypophosphatemia		
		Tumor lysis syndrome	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		
NERVOUS SYSTEM DISORDERS			
	Headache		***Headache (Gr 2)***
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Cough		***Cough (Gr 2)***
VASCULAR DISORDERS			
	Hypertension		

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

Adverse events reported on venetoclax (ABT-199) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that venetoclax (ABT-199) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Hemolysis; Thrombotic thrombocytopenic purpura
CARDIAC DISORDERS - Atrial fibrillation; Cardiac disorders - Other (coronary artery disease); Heart failure; Myocardial infarction; Sinus tachycardia; Ventricular arrhythmia
EAR AND LABYRINTH DISORDERS - Tinnitus; Vertigo
GASTROINTESTINAL DISORDERS - Abdominal pain; Belching; Dry mouth; Dyspepsia; Dysphagia; Gastrointestinal disorders - Other (Crohn's disease); Small intestinal obstruction
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Death NOS; Edema limbs; Flu like symptoms; General disorders and administration site conditions - Other (general physical heath deterioration); General disorders and administration site conditions - Other (multiple organ dysfunction syndrome); Injection site reaction; Non-cardiac chest pain; Pain; Sudden death NOS

HEPATOBILIARY DISORDERS - Hepatobiliary disorders - Other (hepatic function abnormal)
IMMUNE SYSTEM DISORDERS - Cytokine release syndrome
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Infusion related reaction; Injury, poisoning and procedural complications - Other (laceration)
INVESTIGATIONS - Aspartate aminotransferase increased; Blood bilirubin increased; Weight loss
METABOLISM AND NUTRITION DISORDERS - Anorexia; Dehydration; Hyperglycemia; Hyperkalemia; Hyperphosphatemia; Hyperuricemia; Hypoalbuminemia; Hypomagnesemia; Hyponatremia; Metabolism and nutrition disorders - Other (failure to thrive)
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Back pain; Bone pain
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Myelodysplastic syndrome; Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (acute myeloid leukemia); Treatment related secondary malignancy
NERVOUS SYSTEM DISORDERS - Dizziness; Dysesthesia; Intracranial hemorrhage; Ischemia cerebrovascular; Nervous system disorders - Other (neuropathy peripheral); Peripheral sensory neuropathy; Syncope
PSYCHIATRIC DISORDERS - Confusion; Insomnia
RENAL AND URINARY DISORDERS - Acute kidney injury; Cystitis noninfective; Dysuria
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Ovarian rupture
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Allergic rhinitis; Aspiration; Dyspnea; Epistaxis; Hypoxia; Nasal congestion; Oropharyngeal pain; Pleural effusion; Respiratory failure; Respiratory, thoracic and mediastinal disorders - Other (asphyxia)
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Eczema; Pruritus; Rash acneiform; Rash maculo-papular
VASCULAR DISORDERS - Hypotension; Thromboembolic event

Note: Venetoclax (ABT-199) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

10.1.1.2 CAEPR for ASTX727

Comprehensive Adverse Events and Potential Risks list (CAEPR) for ASTX727 (Cedazuridine, Decitabine, NSC 820631)

The Comprehensive Adverse Events and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 542 patients.* Below is the CAEPR for ASTX727 (cedazuridine and decitabine).

> **NOTE**: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Version 2.1, June 27, 2022[1]

Adverse Events with Possible Relationship to ASTX727 (cedazuridine and decitabine) (CTCAE 5.0 Term) [n= 274]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Anemia			***Anemia (Gr 2)***
	Febrile neutropenia		***Febrile neutropenia (Gr 2)***
GASTROINTESTINAL DISORDERS			
	Constipation		***Constipation (Gr 2)***
	Diarrhea		***Diarrhea (Gr 2)***
	Mucositis oral		
	Nausea		***Nausea (Gr 2)***
	Vomiting		
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
Fatigue			***Fatigue (Gr 2)***
INFECTIONS AND INFESTATIONS			
	Infection[2]		
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			
	Bruising		
INVESTIGATIONS			
	Alanine aminotransferase increased		
Neutrophil count decreased			***Neutrophil count decreased (Gr 2)***
Platelet count decreased			***Platelet count decreased (Gr 2)***
	White blood cell decreased		***White blood cell decreased (Gr 2)***
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		***Anorexia (Gr 2)***
NERVOUS SYSTEM DISORDERS			
	Dizziness		
	Headache		

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

Adverse events reported on ASTX727 (cedazuridine, decitabine) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that ASTX727 (cedazuridine, decitabine) caused the adverse event:

CARDIAC DISORDERS - Heart failure; Myocarditis
GASTROINTESTINAL DISORDERS - Abdominal pain; Colitis
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS – Chills; Edema limbs; Fever
IMMUNE SYSTEM DISORDERS - Allergic reaction
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Fall
INVESTIGATIONS – Alanine aminotransferase increased; Aspartate aminotransferase increased; Blood bilirubin increased; Creatinine increased; Weight loss
METABOLISM AND NUTRITION DISORDERS - Hypoalbuminemia; Hypomagnesemia; Tumor lysis

syndrome
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthralgia; Back pain; Myalgia
PSYCHIATRIC DISORDERS - Insomnia
RENAL AND URINARY DISORDERS- Acute kidney injury
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Adult respiratory distress syndrome; Cough; Epistaxis; Oropharyngeal pain; Pharyngeal hemorrhage; Respiratory failure
SKIN AND SUBCUTANEOUS TISSUE DISORDERS – Pruritis; Rash maculo-papular; Skin and subcutaneous tissue disorders - Other (angioedema)

Note: ASTX727 (cedazuridine, decitabine) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

10.1.2 Adverse Event Lists for Commercial Agents

10.1.2.1 Cytarabine

Common AEs (occurring in greater than 30%) for patients taking cytarabine:

Headache
Low blood counts
Nausea and vomiting
Mouth sores
Increases in blood tests measuring liver function

Less common side effects (occurring in about 10-29%) for patients receiving cytarabine:

Diarrhea
Loss of appetite
Skin rash, redness and itching
Flu-like symptoms
Pain, redness and skin peeling of the palms of hands and soles of feet (hand-foot syndrome)
Temporary hair loss
Eye pain, tearing, sensitivity to light and blurred vision with high-dose therapy

Please refer to the package insert for cytarabine for a comprehensive list of AEs.

10.1.2.2 Daunorubicin

Common AEs (occurring in greater than 30%) for patients taking daunorubicin:

Pain along the site where the medication was given
Urine may appear red, red-brown, orange or pink from the color of the medication.
Low blood counts

Nausea or vomiting
Mouth sores
Hair loss on the scalp or elsewhere on the body

Less common (occurring in 10-29%) for patients taking daunorubicin:

Diarrhea
Problems with fertility
Darkening of skin at the site of previous radiation therapy
Darkening, discoloration of nail beds

Please refer to the package insert for daunorubicin for a comprehensive list of AEs.

10.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **For expedited reporting purposes only:**
  - AEs for the agent that are ***bold and italicized*** in the CAEPR (*i.e.*, those listed in the SPEER column, Section 10.1) should be reported through CTEP-AERS only if the grade is above the grade provided in the SPEER.
  - Other AEs for the protocol that do not require expedited reporting are outlined in Section 10.3.4.

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

10.3 Expedited Adverse Event Reporting

10.3.1 Rave-CTEP-AERS Integration

The Rave Cancer Therapy Evaluation Program Adverse Event Reporting System (CTEP-AERS) integration enables evaluation of Adverse Events (AEs) entered in Rave to determine whether they require expedited reporting and facilitates entry in CTEP-AERS for those AEs requiring expedited reporting. Sites must initiate all AEs for this study in Medidata Rave.

Pre-treatment AEs: AEs that occur after informed consent is signed and prior to start of treatment are collected in Medidata Rave using the Pre-treatment Adverse Event form.

Pre-existing medical conditions (formerly referred to as baseline AEs) identified during baseline assessment are not considered AEs and therefore should not be reported on the Pre-treatment Adverse Event form. If these pre-existing conditions worsen in severity, the investigator must reassess the event to determine if an expedited report is required. Whether or not an expedited report is required, the worsened event should be reported as a routine AE.

Treatment-emergent AEs: All AEs that occur after start of treatment are collected in Medidata Rave using the Adverse Event form, which is available for entry at each treatment course or reporting period and is used to collect AEs that start during the period or persist from the previous reporting period. AEs that occur 30 Days after the Last Administration of the Investigational Agent/Intervention are collected using the Late Adverse Event form.

Prior to sending AEs through the rules evaluation process, site staff should verify the following on the Adverse Event form in Rave:

- The reporting period (course/cycle) is correct, and
- AEs are recorded and complete (no missing fields) and the form is query-free.

The CRA reports AEs in Rave at the time the Investigator learns of the event. If the CRA modifies an AE, it must be re-submitted for rules evaluation.

Upon completion of AE entry in Medidata Rave, the CRA submits the AE for rules evaluation by completing the Expedited Reporting Evaluation form. Both NCI and protocol-specific reporting rules evaluate the AEs submitted for expedited reporting. A report is initiated in CTEP-AERS using information entered in Medidata Rave for AEs that meet reporting requirements. The CRA completes the report by accessing CTEP-AERS via a direct link on the Medidata Rave Expedited Reporting Evaluation form. Contact the CTSU Help Desk at 1-888-823-5923 or by email at ctsucontact@westat.com if you have any issues submitting an expedited report in CTEP-AERS.

In the rare occurrence that internet connectivity is lost, a 24-hour notification is to be made to CTEP by telephone at 301-897-7497. Once internet connectivity is restored, the 24-hour notification that was phoned in must be entered immediately into CTEP-AERS using the direct link from Medidata Rave.

Additional information about the CTEP-AERS integration is available on the CTSU members' website:

- Study specific documents: *Protocols > Documents> Protocol Related Documents> Adverse Event Reporting*, and

- Additional resources: *Resources > CTSU Operations Information> User Guides & Help Topics*.

NCI requirements for SAE reporting are available on the CTEP website:

- NCI Guidelines for Investigators: Adverse Event Reporting Requirements is available at https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf.

10.3.2 Distribution of Adverse Event Reports

CTEP-AERS is programmed for automatic electronic distribution of reports to the following individuals: Principal Investigator and Adverse Event Coordinator(s) (if applicable) of the Corresponding Organization or Lead Organization, the local treating physician, and the Reporter and Submitter. CTEP-AERS provides a copy feature for other e-mail recipients.

10.3.3 Expedited Reporting Guidelines

Use the NCI protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

Note: A death on study requires both routine and expedited reporting, regardless of causality as long as the death occurred within 30 days after the last administration of the investigational agent. Attribution to treatment or other cause must be provided.

Death due to progressive disease should be reported as **Grade 5 "Disease progression"** in the system organ class (SOC) "General disorders and administration site conditions." Evidence that the death was a manifestation of underlying disease (*e.g.*, radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

Phase 1 and Early Phase 2 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention [1, 2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria MUST be immediately reported to the NCI via electronic submission within the timeframes detailed in the table below.

Hospitalization	Grade 1 and Grade 2 Timeframes	Grade 3-5 Timeframes
Resulting in Hospitalization ≥ 24 hrs	10 Calendar Days	24-Hour 5 Calendar Days
Not resulting in Hospitalization ≥ 24 hrs	Not required	

NOTE: Protocol specific exceptions to expedited reporting of serious adverse events are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR.

Expedited AE reporting timelines are defined as:

- "24-Hour; 5 Calendar Days" - The AE must initially be submitted electronically within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
- "10 Calendar Days" - A complete expedited report on the AE must be submitted electronically within 10 calendar days of learning of the AE.

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:

Expedited 24-hour notification followed by complete report within 5 calendar days for:

- All Grade 3, 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

[2]For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

Effective Date: May 5, 2011

10.3.4 Additional Protocol-Specific Expedited Adverse Event Reporting Exclusions

For this protocol only, the AEs/grades listed below do not require expedited reporting via CTEP-AERS. However, they still must be reported through the routine reporting mechanism (Section 10.4):

CTCAE SOC	Adverse Event	Grade	≥24h Hospitalization[a]
Blood and lymphatic system disorders	Febrile neutropenia	≤3	Regardless
Blood and lymphatic system disorders	Thrombocytopenia	≤3	Regardless

[a] Indicates that an adverse event required hospitalization for ≥24 hours or prolongation of hospitalization by ≥24 hours of a patient.

10.4 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions. **AEs reported expeditiously through CTEP-AERS must also be reported in routine study data submissions.**

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. AEs are reported in a routine manner at scheduled times during the trial using Medidata Rave. For this trial the Adverse Event CRF is used for routine AE reporting in Rave.

10.5 Pregnancy

Although not an adverse event in and of itself, pregnancy as well as its outcome must be documented via **CTEP-AERS**. In addition, the ***Pregnancy Information Form*** included within the NCI Guidelines for Adverse Event Reporting Requirements must be completed and submitted to CTEP. Any pregnancy occurring in a patient or patient's partner from the time of consent to 90 days after the last dose of study drug must be reported and then followed for outcome. Newborn infants should be followed until 30 days old. Please see the "NCI Guidelines for Investigators: Adverse Event Reporting Requirements for DCTD (CTEP and CIP) and DCP INDs and IDEs" (at http://ctep.cancer.gov/protocolDevelopment/adverse_effects.htm) for more details on how to report pregnancy and its outcome to CTEP.

10.6 Secondary Malignancy

A *secondary malignancy* is a cancer caused by treatment for a previous malignancy (*e.g.*, treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.

CTEP requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported expeditiously via CTEP-AERS. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy (*e.g.*, acute myelocytic leukemia [AML])
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

10.7 Second Malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy). Second malignancies require **ONLY** routine AE

reporting unless otherwise specified.

11. STUDY CALENDAR

Baseline (screening) evaluations are to be conducted within 2 weeks prior to start of protocol therapy. Scans and x-rays must be done ≤4 weeks prior to the start of therapy. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

11.1 Study Calendar for Venetoclax+ASTX727 Combination *(Note: The Phase 1b portion of the trial is now closed. Moving forward, all new patients will be enrolled in the Phase II portion of the trial as per Amendment #04 (July 13, 2022).*

Protocol Activities Cycle = 28days[a]	Baseline/ Screening (Day -14 to Day -1	CYCLE 1									CYCLES2+									Post-Treatment	
		D1	D2	D3	D4	D5	D8 +/- 2 days	D15	D22	D28	D1 +/- 2 days	D2	D3	D4	D5	D8	D15	D22	D28	EOT[r]	Follow-Up[s]
Venetoclax[p]		X----------								----X	X----------								----X		
ASTX727[p]		X	X	X	X	X					X	X	X	X	X						
Informed consent[b]	X																				
Demographics	X																				
Medical history	X																				
Concurrent meds and adverse events[d]	X	X	X	X	X	X	X	X	X		X									X	X
Physical exam	X	X					X	X	X		X									X	X
ECOG Performance status	X	X									X									X	X
Vital signs[c]	X	X	X	X	X	X	X	X	X		X									X	X
Height	X																				
Weight	X	X					X	X	X		X									X	X
DLT evaluation (phase 1b only)[e]		X	X	X	X	X	X	X	X		X									X	
Survival follow-up[t]																					X
CBC w/diff, [q]	X	X	X	X	X	X	X	X	X		X									X	X
Comprehensive Chemistry Panel·[q]	X	X	X	X	X	X	X	X	X		X									X	X
Mg, Phos[q]	X	X	X	X	X	X	X	X	X		X									X	
TLS labs[q]	X	X	X	X	X	X	X	X	X		X										
LDH, uric acid[q]	X	X	X	X	X	X	X	X	X		X									X	

Protocol Activities Cycle = 28days[a]	Baseline/ Screening (Day -14 to Day -1	CYCLE 1									CYCLES2+									Post-Treatment	
		D1	D2	D3	D4	D5	D8 +/- 2 days	D15	D22	D28	D1 +/- 2 days	D2	D3	D4	D5	D8	D15	D22	D28	EOT[r]	Follow-Up[s]
Coagulation testing (PT/INR, PTT)	X																			X	
12-lead ECG[h]	X																			X	
Pregnancy test[g]	X	X																		X	
Urinalysis[f]	X																			X	
Chest radiograph[i]	X																			X	
Bone marrow aspiration for disease assessment[j]	X										X									X	
Bone marrow aspiration for research collections[k]	X						X													X	
Blood collection in EDTA tubes for Pharmocokinetics[l]						X			X												
IVF/Allopurinol[m]	X	X----------X																			
Antimicrobial prophylaxis[n]	X	X----------X																			
Anti-emetic oral administration[o]		X	X	X	X	X															

Protocol Activities Cycle = 28days[a]	Baseline/ Screening (Day -14 to Day -1	CYCLE 1									CYCLES2+									Post-Treatment	
		D1	D2	D3	D4	D5	D8 +/- 2 days	D15	D22	D28	D1 +/- 2 days	D2	D3	D4	D5	D8	D15	D22	D28	EOT[r]	Follow-Up[s]

a. Cycle length will typically be 28-42 days as per DEC prescribing information instructions. However, initial cycles with venetoclax+DNMTi have been considerably longer in some patients, and investigators will monitor marrow function closely prior to subsequent cycles of ASTX727. Instructions on venetoclax dosing interruption are in the protocol.

b. Informed consent must be obtained before any study-specific screening assessments are performed. Screening assessments are to be performed within 14 days prior to Day 1 of Cycle 1 unless otherwise noted. Assessments performed as standard of care within the screening window may be used for screening. Baseline characteristics include but are not limited to: demographics, medical and surgical history, extent of disease, prior anti-cancer treatment, and disease morphology.

c. Vital Signs to include: Blood pressure (BP), heart rate (HR) and temperature. To be done at least once on days indicated (required pre-dose on Cycle 1, Day; and thereafter, ideally prior to any receipt of ASTX727 or venetoclax on the indicated day), with any additional recordings per local site's standard of care / treating physician discretion.

d. Review and capture of all concomitant medications will be performed as indicated. Concomitant medications include prescription medications and over-the-counter preparations (including vitamins, dietary supplements, over-the-counter medications, and herbal preparations) used by a patient within at least 14 days prior to first dose of protocol-defined treatment and continuing through at least the 28-day Follow-Up study visit. After signing informed consent, adverse events will be collected as detailed in protocol. All adverse events will be followed at least until 28 days after a patient's final protocol-directed treatment with ASTX727 or venetoclax (whichever occurs last) or until initiation of another anti-cancer therapy – whichever occurs first.

e. For Phase 1b only: Dedicated surveillance and expedited reporting of Dose Limiting Toxicity (DLT) required for 28 days after initiating protocol-indicated treatment. On Cycle 2, Day 1 (i.e. overall Day 29), satisfactory completion of the physical exam and safety labs is required to help detect evidence of past or present DLT as having occurred during the first 28 days of protocol-indicated treatment. If intolerable delayed toxicity attributable (per sponsor-investigator judgement) to protocol treatment is detected past Day 28, then the 28-Day DLT window may possibly be extended. See protocol Section 6.2 for definition of DLT and toxicities which, if experienced during the first 28 days after initiating protocol-indicated treatment, shall be considered dose limiting. DLT evaluation should be on C2D1 and without allowance of a 2-day window

f. Macroscopic urinalysis to include specific gravity, pH, glucose, protein, ketones, and blood. Increased frequency of urinalysis and urine microscopies (e.g. RBC, WBC, casts, and crystals) as clinically indicated per patient's study physician.

g. For women of childbearing potential (WOCBP): Serum- or urine-based pregnancy test required during Screening; and either serum or urine pregnancy test required ≤ 3 days prior to first dose of protocol-indicated treatment (if test during Screening was done > 3 days prior to initiating c1d1 treatment), and at End-of-Treatment. A woman of childbearing potential is defined as any female who has experienced menarche who has not undergone surgical sterilization (i.e. bilateral tubal ligation, total hysterectomy, or bilateral oophorectomy) at least 4 weeks prior to first dose of protocol-indicated treatment, or who is not postmenopausal. Menopause is defined clinically as 12 months of amenorrhea in a woman over 45 in the absence of other biological or physiological causes.
If patient's postmenopausal status is considered for childbearing potential and study-required contraception then postmenopausal status in females < 62 years of age must be confirmed at baseline (prior to initiating protocol-indicated treatment) with a serum follicle-stimulating hormone (FSH) level within laboratory reference range for postmenopausal women.

h. One standard 12-lead electrocardiogram (ECG) using local site equipment during Screening and EOT visits. Additional ECGs as clinically indicated per study protocol or patient's study physician.

i. Chest X-ray (both posteroanterior and lateral films) required ≤ 28 days prior to first dose of protocol-indicated treatment, and at EOT only if pulmonary symptoms arise on treatment (unless Chest X-ray was already done ≤ 28 days prior to the EOT visit). Alternatively, chest CT also acceptable – if Chest CT is already available as a standard-of-care scan done ≤ 28 days prior to first dose of study treatment / EOT.

j. Standard of care bone marrow aspiration and biopsy are required at screening prior to enrollment, C2D1(+/- 3 days), C5D1(+/- 3 days), and C8D1(+/- 3 days). Bone marrow aspiration to assess disease progression is required unless clear evidence of progressive disease (eg. >20% blasts in peripheral blood, myeloid sarcoma, or leukemia cutis). Note that marrow biopsy/aspirate is **NOT** required to be repeated as an EoT activity if already performed at recovery. Molecular (mutation testing) and cytogenetic (classic karyotyping testing ± FISH) will be performed as per standard of care. These analyses should be reported at study enrollment and in setting of response assessment at recovery or EoT, per standard of care. Disease assessment via bone marrow biopsy and aspiration and survival will be noted during therapy and at C5D1 and C8D1 as above. During therapy, disease assessment should be reported in setting of response assessment at recovery or EoT.

k. Bone marrow aspirations required for research samples. At screening/baseline and on **C1D8 (venetoclax/ASTX727 arm only)**, bone marrow aspirate for research is required. For the screening/baseline aspiration, patients must have at least >/=15 mL, or $20x10^7$ MNCs, whichever is greater, from bone marrow aspirate obtained within 21 days of beginning treatment on this study. Patients who consent to undergo fresh biopsy prior to initiating this study treatment, but whose disease is deemed inaccessible (or insufficient) to a reasonably safe biopsy, may be allowed to enroll upon discussion with and approval by the principal investigator (if otherwise eligible and with documented disease pathology meeting inclusion criteria). Prior to initiating treatment, a patient's screening team must have documentation that archival or fresh biopsy tissue has been requested and that a reasonable attempt has been made to obtain such

Protocol Activities Cycle = 28days[a]	Baseline/ Screening (Day -14 to Day -1	CYCLE 1									CYCLES2+									Post-Treatment	
		D1	D2	D3	D4	D5	D8 +/- 2 days	D15	D22	D28	D1 +/- 2 days	D2	D3	D4	D5	D8	D15	D22	D28	EOT[r]	Follow-Up[s]

biospecimen for exploratory research. (Provided the diagnosis of eligible disease meeting inclusion criteria is not in question, waiting for histological analysis or confirmation that acquired specimens are known to contain tissue sufficient for exploratory analysis is not a requirement prior to initiating study treatment). For the C1D8 aspiration (venetoclax/ASTX727 arm only), at least >/=15 mL, or $10x10^7$ MNCs, whichever is greater, from bone marrow aspirate is required. For the optional EOT aspirate, at least >/=15 mL, or $20x10^7$ MNCs, whichever is greater, from bone marrow aspirate is required.

l. Pharmacokinetic studies will be conducted on C1D5 and C1D22+/- 2 days at before venetoclax, 1 hour, 2hour, 4hour, and 7 hours after venetoclax.

m. Anti-TLS hydration/prophylaxis (oral / i.v.): During cycle 1 of venetoclax+ASTX727, TLS precautions will include daily visits with TLS laboratory studies and pre-emptive therapy with a uric acid reducing agent (i.e., allopurinol or febuxostat). Laboratory monitoring for TLS will occur prior to the first dose on cycle 1 day 1, 6-8 hours after, at 24 ± 4 hours after the first dose and daily until WBC is less than 5,000 cells/uL, or first 5 days of therapy, whichever is longer. Allopurinol should be initiated at least 2-3 days prior to the start of therapy and continued through the end of cycle 1 if no clinically significant TLS. In the event of inability to maintain copious fluid intake, intravenous fluids should be administered daily during C1. If there is clinical evidence of TLS in the opinion of the investigator(s), hospitalization should be considered.

n. Antimicrobial prophylaxis may begin when ANC is below 500 with antibacterial and antiviral prophylaxis. Antifungal prophyaxis may be given in accordance with local standards of practice and after C1D14 as described within section 6.3.2.1 of the protocol with consideration of CYP450-mediated concomitant medication risks.

o. To minimize nausea, unless contraindicated, all patients in Phase 1b and Phase 2 must receive a 5-HT3 antagonist (e.g. ondansetron 4 mg or equivalent) starting at least 1 hour before patient's first dose of ASTX727. Alternative anti-emetic agent(s) may be used if the patient does not tolerate a 5-HT3 antagonist. After the patient completes the first 5 days of therapy with ASTX727, use of an anti-emetic agent is at the discretion of the patient's study physician.

p. Beginning on Cycle 1, Day 1 (after receiving mandatory anti-TLS hydration/prophylaxis – see Note l above, patients randomized to the venetoclax/ASTX727 arm are scheduled to initiate oral venetoclax once-a-day (QD) on a continuous daily dosing schedule. ASTX727 will be offered in a de-escalation design by dosing days. This will start at D1-5 then D1-4, and finally D1,3, 5, if necessary. Note: On days involving dosing of both ASTX727 and venetoclax, the order of ASTX727/venetoclax administration is not specified – i.e. either oral drug, ASTX727or venetoclax, may be taken before or after the other drug

q. On Days 2, 3, 4, 5, 8, 15, 22, of Cycle 1 safety labs required. These include: Hematology (white blood cell count with differential, hemoglobin, hematocrit, and platelet count); Blood Chemistries (sodium, potassium, chloride, bicarbonate or carbon dioxide, blood urea nitrogen, creatinine, glucose, total bilirubin, AST, ALT, alkaline phosphatase, calcium, albumin, and total protein); Mg, Phos; and additional TLS labs, LDH, and Uric Acid. Additionally, electrolytes will be measured twice within week 1 of cycle 2. TLS should be monitored at pre-dose, 6 to 8 hours after each new dose during ramp-up, and 24 hours after reaching final dose. These same laboratories also to be obtained more often in the cycle, if clinically indicated per patient's study physician.

r. Reasonable effort should be made to complete End-of-Treatment (EOT) procedures on the day it is decided a patient will no longer receive protocol-indicated treatment. These procedures must be completed subsequent to and not later than 10 days after investigator decision to permanently discontinue protocol-indicated treatment with ASTX727 or venetoclax (whichever treatment occurs last) and prior to any subsequent anti-cancer therapy. If patient discontinues study treatment for reason other than progressive disease confirmed by biopsy procedure (e.g. due to adverse event), then every effort should be made to obtain fresh biopsy samples to evaluate response and to obtain biospecimens for exploratory research. If progressive disease is suspected between scheduled study biopsies (e.g. due to suspicious peripheral blood counts), then fresh biopsy samples must be collected in a subsequent procedure to evaluate for progressive disease and to obtain biospecimens for research purposes; if progressive disease is confirmed in that procedure and the patient will terminate the study as a result, then such procedure can be considered synonymous with patient's biopsy procedure required at end-of-treatment.

s. Follow-up clinic visit to be completed 28 days (+7 days) after patient's final protocol-indicated treatment with ASTX727 or venetoclax (whichever occurs last). Documented attempt(s) must be made for patient return to the study clinic. It will not be considered a protocol deviation if the patient is physically unable to return for the follow-up visit; such circumstance should be recorded in the study documents, and as much of the follow-up information as possible should be obtained via feasible patient contact and from local and outside facilities.

t. Each patient will be followed for survival every 3 months (± 14 days) after patient's final protocol treatment with ASTX727 or venetoclax (whichever occurs last) until death, end of the study, until patient withdraws consent, or for a maximum of 2 years after the patient's final protocol-indicated treatment – whichever comes first. Survival contact can be made via clinic visit, chart review, obituary or similar observation (e.g. Social Security death index), or by telephone.

11.2 Study Calendar for 7+3 Control Treatment Arm

Protocol Activities Cycle = 4 weeks (28 days)	Baseline/ Screening (Day -14 to Day -1	CYCLE 1 Induction					Cycle 1 response assessments	CYCLE 2 Induction				Cycle 2 response assessments	Post-Treatment	
		D1	D3	D4	D5	D7	Weekly until recovery or day 57 +/- 3 days[h,i]	D1	D2	D4	D5	Weekly until recovery or day 57 +/- 3 days[h,i]	EOT[o]	Follow-Up (28d + 7d after last study dose)[p]
Cytarabine[j]		X----------X						X----------X						
Daunorubicin[k]		X------X						X---------X						
Informed consent[a]	X													
Demographics	X													
Medical history	X													
Concurrent meds and adverse events[c]	X	X		X			X	X			X	X	X	X
Physical exam[b]	X	X		X			X	X			X	X	X	X
ECOG Performance Status[b]	X	X					X	X				X	X	X
Vital signs[b]	X	X		X			X	X			X	X	X	X
Height	X													
Weight[b]	X	X		X			X	X			X	X	X	X
Survival follow-up[q]														X
CBC w/diff, plts[e]	X	X		X			X	X			X	X	X	X
Comprehensive Chemistry Panel[f]	X	X		X			X	X			X	X	X	X
Coagulation testing (PT/INR, PTT)	X												X	
12-lead ECG	X													
Echocardiogram[d]	X													
Pregnancy test[g]	X													
Assessment of cardiac ejection fraction (EF%)	X							X						

Protocol Activities Cycle = 4 weeks (28 days)	Baseline/ Screening (Day -14 to Day -1	CYCLE 1 Induction					Cycle 1 response assessments	CYCLE 2 Induction				Cycle 2 response assessments	Post-Treatment	
		D1	D3	D4	D5	D7	Weekly until recovery or day 57 +/- 3 days[h,i]	D1	D2	D4	D5	Weekly until recovery or day 57 +/- 3 days[h,i]	EOT[o]	Follow-Up (28d + 7d after last study dose)[p]
Urinalysis	X												X	
Chest radiograph[j]	X													
Bone marrow aspiration and biopsy for disease status[h]	X						X					X	X	
Bone marrow aspitation for research[i]	X												X	
IVF/Allopurinol[l]		X----------X												
Antimicrobial prophylaxis[m]		X----------X												
Anti-emetic oral administration[n]		X----------X												

a Informed consent must be obtained within 14 days prior to initiation of trial treatment

b The screening physical examination, ECOG performance status, hematology, chemistry, PT/INR and serum or urine pregnancy test must be conducted within 7 days from C1D1. If the initial examinations are obtained within 72 hours of C1D1, they do not need be repeated. At the baseline visit only, height will also be recorded. PEs and laboratory assessments will be completed until count recovery as directed on calendar. Directed physical exams as per investigator discretion. Vital signs to include weight.

c Adverse event reporting will be required on days as noted in schedule of assessments. In the event that AEs occur between these visits, they will be captured if they meet criteria as defined in section 10.

d Echocardiogram will be conducted to assess cardiac function prior to administration of chemotherapy. In the event that there are abnormalities on the initial echocardiogram, or if the investigator has concern for cardiac complications during the first induction, a second echocardiogram may be conducted as per standard of practice.

e CBC with differential will include RBC, Hgb, hematocrit, total WBC, ANC, platelet count, and differential

f Chemistry panel includes the following: urea or BUN, creatinine, sodium, potassium, calcium, magnesium, phosphorus, glucose albumin, total protein, ALT, AST, total bilirubin, uric acid, and LDH

g A serum- or urine-based pregnancy test will be performed only at baseline when appropriate as per protocol..

h Standard of care bone marrow aspiration and biopsy are required at screening prior to enrollment, and end of treatment (EOT). EOT assessment should occur between D28-57 after last induction (1st or 2nd). Bone marrow aspiration to assess disease progression is optional. Note that marrow biopsy/aspirate is **NOT** required to be repeated as an EOT activity if already performed at recovery. Molecular (mutation testing) and cytogenetic (classic karyotyping testing ± FISH) will be performed as per standard of care. These analyses should be reported at study enrollment and in setting of response assessment at recovery or EoT, per standard of care. Disease assessment via bone marrow biopsy and aspiration and survival will be noted during therapy as above. During therapy, disease assessment should be reported in setting of response assessment at recovery or EoT. Mid cycle bone marrow biopsy (Day 14-Day 21) will be done per SOC and will be used to determine if a second induction cycle is required. Mid cycle bone marrow biopsy is required if additional treatment will be given.

i Bone marrow aspirations required for research samples. At screening/baseline a bone marrow aspirate for research is required. For the screening/baseline aspiration, patients must have at least >/=15 mL, or $20x10^7$ MNCs, whichever is greater, from bone marrow aspirate obtained within 21 days of beginning treatment on this study. Patients who consent to undergo fresh biopsy prior to initiating this study treatment, but whose disease is deemed inaccessible (or insufficient) to a reasonably safe biopsy, may be allowed to enroll upon discussion with and approval by the principal investigator (if otherwise eligible and with documented disease pathology meeting inclusion criteria). Prior to initiating treatment, a patient's screening team must have documentation that archival or fresh biopsy tissue has been requested and that a reasonable attempt has been made to obtain such biospecimen for exploratory research. (Provided the diagnosis of eligible disease meeting inclusion criteria is not in question, waiting for histological analysis or confirmation that acquired specimens are known to contain tissue sufficient for exploratory analysis is not a requirement prior to initiating study treatment). For the optional EOT aspirate, at least >/=15 mL, or $20x10^7$ MNCs, whichever is greater, from bone marrow aspirate is required. EoT activities required ONLY if disease assessment procedures not already performed in setting of count recovery during *response assessment period* (C1 and/or C2). See also footnote h..

j Cytarabine infusion at $100mg/m^2$ on days 1,2,3,4,5,6,7 of 1st induction cycle and days 1,2,3,4,5 of 2nd induction cycle .

k Daunorubicin infusion at $60mg/m^2$ on days 1,2,3 of 1st inductioncycle and on days 1.2 of 2nd induction cycle.

Protocol Activities Cycle = 4 weeks (28 days)	Baseline/ Screening (Day -14 to Day -1	CYCLE 1 Induction					Cycle 1 response assessments	CYCLE 2 Induction				Cycle 2 response assessments	Post-Treatment	
		D1	D3	D4	D5	D7	Weekly until recovery or day 57 +/- 3 days[h,i]	D1	D2	D4	D5	Weekly until recovery or day 57 +/- 3 days[h,i]	EOT[o]	Follow-Up (28d + 7d after last study dose)[p]

l Allopurinol to start ≥ 48h prior to C1D1 and continue through C1, and then at the discretion of investigator. IVF to be given as indicated by protocol, and per treating investigator.
m Antimicrobial prophylaxis shall begin when ANC is below 500. Antibacterial, antiviral and antifungal prophylaxis will be given in accordance with the protocol and consideration of CYP450-mediated concomitant medication risks as noted in protocol.
n To minimize nausea, unless contraindicated, all patients in Dose-Escalation and expansion phases must receive a 5-HT3 antagonist (*e.g*. ondansetron 16 mg or equivalent) starting at least 1 hour before patient's first dose of daunorubicin. Alternative anti-emetic agent(s) may be used if the patient does not tolerate a 5-HT3 antagonist. After the patient completes the first 3 days of therapy with daunorubicin, use of an anti-emetic agent is at the discretion of the patient's study physician.
o Reasonable effort should be made to complete End-of-Treatment (EOT) procedures on the day it is decided a patient will no longer receive protocol-indicated treatment. These procedures must be completed subsequent to and not later than 10 days after investigator decision to permanently discontinue protocol-indicated treatment with cytarabine or daunorubicin (whichever treatment occurs last) and prior to any subsequent anti-cancer therapy. If patient discontinues study treatment for reason other than progressive disease confirmed by biopsy procedure (e.g. due to adverse event), then every effort should be made to obtain fresh biopsy samples to evaluate response and to obtain biospecimens for exploratory research. If progressive disease is suspected between scheduled study biopsies (e.g. due to suspicious peripheral blood counts), then fresh biopsy samples must be collected in a subsequent procedure to evaluate for progressive disease and to obtain biospecimens for research purposes; if progressive disease is confirmed in that procedure and the patient will terminate the study as a result, then such procedure can be considered synonymous with patient's biopsy procedure required at end-of-treatment.
p Follow-up clinic visit to be completed 28 days (+7 days) after patient's final protocol-indicated treatment with cytarabine or daunorubicin (whichever occurs last). Documented attempt(s) must be made for patient return to the study clinic. It will not be considered a protocol deviation if the patient is physically unable to return for the follow-up visit; such circumstance should be recorded in the study documents, and as much of the follow-up information as possible should be obtained via feasible patient contact and from local and outside facilities.
q Each patient will be followed for survival every 3 months (± 14 days) after patient's final protocol treatment with cytarabine or daunorubicin (whichever occurs last) until death, end of the study, until patient withdraws consent, or for a maximum of 2 years after the patient's final protocol-indicated treatment – whichever comes first. Survival contact can be made via clinic visit, chart review, obituary or similar observation (e.g. Social Security death index), or by telephone.

12. MEASUREMENT OF EFFECT

12.1 Definitions

Evaluable for toxicity:
As patients who receive protocol directed therapy will be evaluable for toxicity.

Evaluable for objective response.
Phase 2 patients are unevaluable only if untreated.

12.2 Antitumor Effect – Hematologic Tumors

12.2.1 **Complete Response (CR) without minimal residual disease (CRMRD−):** If studied pretreatment, CR with negativity for a genetic marker by RT-qPCR, or CR with negativity by MFC

12.2.2 **Complete remission (CR):** Bone marrow blasts <5%; absence of circulating blasts and blasts with Auer rods; absence of extramedullary disease; ANC ≥1.0 × 10^9/L (1000/μL); platelet count ≥100 × 10^9/L (100 000/μL); MRD+ or unknown

12.2.3 **CR with incomplete count recovery (CRi):** All CR criteria except for residual neutropenia (<1.0 × 10^9/L [1000/μL]) or thrombocytopenia (<100 × 10^9/L [100,000/μL]

12.2.4 **CR with partial recovery count (CRh):** All CR criteria with neutrophil count ≥0.5 × 10^9/L and platelet count ≥50 × 10^9/L. *CRh is being recorded as a separate response criteria from other responses (for example patient could have CRi by traditional criteria but also CRh).*

12.2.5 **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%

Peripheral Blood Counts:

- Neutrophil count ≥1.0 × 10^9/L.
- Platelet count ≥100 × 10^9/L.
- Reduced hemoglobin concentration or hematocrit has no bearing on remission status.
- Leukemic blasts must not be present in the peripheral blood.

Bone Marrow Aspirate and Biopsy:

- Cellularity of bone marrow biopsy must be >20% with maturation of all cell lines.
- <5% blasts

- Auer rods must not be detectable.

Extramedullary leukemia, such as CNS or soft tissue involvement, must not be present.

12.2.6 **Stable disease**: Absence of CRMRD−, CR, CRi, PR, MLFS; and criteria for PD not met.

Period of stable disease should last at least 3 months

12.2.7 **Progressive disease (PD)**: Evidence for an increase in bone marrow blast percentage and/or increase of absolute blast counts in the blood

- >50% increase in marrow blasts over baseline (a minimum 15% point increase is required in cases with <30% blasts at baseline; or persistent marrow blast percentage of >70% over at least 3 mo; without at least a 100% improvement in ANC to an absolute level (>0.5 × 10^9/L [500/μL], and/or platelet count to >50 × 10^9/L [50 000/μL] nontransfused)
- >50% increase in peripheral blasts (WBC × % blasts) to >25 × 10^9/L (>25 000/μL) (in the absence of differentiation syndrome)
- New extramedullary disease Some protocols may allow transient addition of hydroxyurea to lower blast counts

"Progressive disease" is usually accompanied by a decline in ANC and platelets and increased transfusion requirement and decline in performance status or increase in symptoms.

12.3 Exploratory Antitumor Effect – Hematologic Tumors

12.3.1 **Hematologic improvement:** Erythroid response (E) (pretreatment Hgb <11 g/dL) Hgb increase by ≥1.5 g/dL, Platelet response (P) (pretreatment platelets <100 × 10^9/L) Absolute increase of ≥30 × 10^9/L for patients starting with >20 × 10^9/L platelets Increase from <20 × 10^9/L to >20 × 10^9/L and by at least 100%.

12.3.2 **Morphologic leukemia-free state (MLFS):** Bone marrow blasts <5%; absence of blasts with Auer rods; absence of extramedullary disease; no hematologic recovery required. (Marrow should not merely be "aplastic"; at least 200 cells should be enumerated or cellularity should be at least 10%)

13. STUDY OVERSIGHT AND DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 10 (Adverse Events: List and Reporting Requirements).

13.1 Study Oversight

This protocol is monitored at several levels, as described in this section. The Protocol Principal Investigator is responsible for monitoring the conduct and progress of the clinical trial, including

the ongoing review of accrual, patient-specific clinical and laboratory data, and routine and serious adverse events; reporting of expedited adverse events; and accumulation of reported adverse events from other trials testing the same drug(s). The Protocol Principal Investigator and statistician have access to the data at all times through the CTMS web-based reporting portal.

All Study Investigators at participating sites who register/enroll patients on a given protocol are responsible for timely submission of data via Medidata Rave and timely reporting of adverse events for that particular study. This includes timely review of data collected on the electronic CRFs submitted via Medidata Rave.

All studies are also reviewed in accordance with the enrolling institution's data safety monitoring plan.

13.2 Data Reporting

Medidata Rave is a clinical data management system being used for data collection for this trial/study. Access to the trial in Rave is controlled through the CTEP-IAM system and role assignments.
Requirements to access Rave via iMedidata:

- A valid account, and
- Assigned a Rave role on the LPO or PO roster at the enrolling site of: Rave CRA, Rave Read Only, Rave CRA (LabAdmin), Rave SLA, or Rave Investigator.
  Rave role requirements:
    - Rave CRA or Rave CRA (Lab Admin) role, must have a minimum of an Associate Plus (AP) registration type,
    - Rave Investigator role, must be registered as an Non-Physician Investigator (NPIVR) or Investigator (IVR), and
    - Rave Read Only role, site staff must have at a minimum an Associates (A) registration type.
- Refer to https://ctep.cancer.gov/investigatorResources/default.htm for registration types and documentation required.

If the study has a DTL, individuals requiring write access to Rave must also be assigned the appropriate Rave tasks on the DTL.

Upon initial site registration approval for the study in Regulatory Support System (RSS), all persons with Rave roles assigned on the appropriate roster will be sent a study invitation email from iMedidata. To accept the invitation, site staff must either click on the link in the email or log in to iMedidata via the CTSU members' website under *Data Management* > *Rave Home* and click to accept the invitation in the Tasks pane located in the upper right-corner of the iMedidata screen. Site staff will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings) and can be accessed by clicking on the eLearning link in the *Tasks* pane located in the upper right corner of the iMedidata screen. If an eLearning is required for a study and has

not yet been taken, the link to the eLearning will appear under the study name in the *Studies* pane located in the center of the iMedidata screen; once the successful completion of the eLearning has been recorded, access to the study in Rave will be granted, and a *Rave EDC* link will replace the eLearning link under the study name.

Site staff that have not previously activated their iMedidata/Rave account at the time of initial site registration approval for the study in RSS will receive a separate invitation from iMedidata to activate their account. Account activation instructions are located on the CTSU website in the Data Management section under the Rave resource materials (Medidata Account Activation and Study Invitation Acceptance). Additional information on iMedidata/Rave is available on the CTSU members' website in the Data Management > Rave section at www.ctsu.org/RAVE/ or by contacting the CTSU Help Desk at 1-888-823-5923 or by e-mail at ctsucontact@westat.com.

13.2.1 Method

This study will be monitored by the Clinical Trials Monitoring Service (CTMS). Data will be submitted to CTMS at least once every two weeks via Medidata Rave (or other modality if approved by CTEP). Information on CTMS reporting is available at http://www.theradex.com/clinicalTechnologies/?National-Cancer-Institute-NCI-11. On-site audits will be conducted three times annually (one annual site visit and two data audits). For CTMS monitored studies, after users have activated their accounts, please contact the Theradex Help Desk at (609) 619-7862 or by email at CTMSSupport@theradex.com for additional support with Rave and completion of CRFs.

13.2.2 Responsibility for Data Submission

For ETCTN trials, it is the responsibility of the PI(s) at the site to ensure that all investigators at the ETCTN Sites understand the procedures for data submission for each ETCTN protocol and that protocol specified data are submitted accurately and in a timely manner to the CTMS via the electronic data capture system, Medidata Rave.

Data are to be submitted via Medidata Rave to CTMS on a real-time basis, but no less than once every 2 weeks. The timeliness of data submissions and timeliness in resolving data queries will be tracked by CTMS. Metrics for timeliness will be followed and assessed on a quarterly basis. For the purpose of Institutional Performance Monitoring, data will be considered delinquent if it is greater than 4 weeks past due.

Data from Medidata Rave and CTEP-AERS is reviewed by the CTMS on an ongoing basis as data is received. Queries will be issued by CTMS directly within Rave. The queries will appear on the Task Summary Tab within Rave for the CRA at the ETCTN to resolve. Monthly web-based reports are posted for review by the Drug Monitors in the IDB, CTEP. Onsite audits will be conducted by the CTMS to ensure compliance with regulatory requirements, GCP, and NCI policies and procedures with the overarching goal of ensuring the integrity of data generated from NCI-sponsored clinical trials, as described in the ETCTN Program Guidelines, which may be found on the CTEP (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm) and

CTSU websites.

CTMS will utilize a core set of eCRFs that are Cancer Data Standards Registry and Repository (caDSR) compliant (http://cbiit.nci.nih.gov/ncip/biomedical-informatics-resources/interoperability-and-semantics/metadata-and-models). Customized eCRFs will be included when appropriate to meet unique study requirements. The PI is encouraged to review the eCRFs, working closely with CTMS to ensure prospectively that all required items are appropriately captured in the eCRFs prior to study activation. CTMS will prepare the eCRFs with built-in edit checks to the extent possible to promote data integrity.

CDUS data submissions for ETCTN trials activated after March 1, 2014, will be carried out by the CTMS contractor, Theradex. CDUS submissions are performed by Theradex on a monthly basis. The trial's lead institution is responsible for timely submission to CTMS via Rave, as above.

Further information on data submission procedures can be found in the ETCTN Program Guidelines (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm).

13.3 Data Quality Portal

The Data Quality Portal (DQP) provides a central location for site staff to manage unanswered queries and form delinquencies, monitor data quality and timeliness, generate reports, and review metrics.

The DQP is located on the CTSU members' website under Data Management. The Rave Home section displays a table providing summary counts of Total Delinquencies and Total Queries. DQP Queries, DQP Delinquent Forms, DQP Form Status and the DQP Reports modules are available to access details and reports of unanswered queries, delinquent forms, forms with current status and timeliness reports. Review the DQP modules on a regular basis to manage specified queries and delinquent forms.

The DQP is accessible by site staff that are rostered to a site and have access to the CTSU website. Staff that have Rave study access can access the Rave study data using a direct link on the DQP.

To learn more about DQP use and access, click on the Help icon displayed on the Rave Home, DQP Queries, DQP Delinquent Forms, DQP Form Status, and DQP Reports modules.

13.4 CTEP Multicenter Guidelines

N/A

13.5 Collaborative Agreements Language

The agent(s) supplied by CTEP, DCTD, NCI used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA, CSA) between the Pharmaceutical Company(ies) (hereinafter referred to as "Collaborator(s)") and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator" (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm) contained within the terms of award, apply to the use of the Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: http://ctep.cancer.gov.

2. For a clinical protocol where there is an investigational Agent used in combination with (an)other Agent(s), each the subject of different Collaborative Agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data"):

    a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NCI, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.

    b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own Agent.

    c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own Agent.

3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order as described in the IP Option to Collaborator (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm). Additionally, all Clinical Data and Results and Raw Data will be collected, used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 C.F.R. Part 164.

4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.

5. Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.

6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group office for Cooperative Group studies or by the principal investigator for non-Cooperative Group studies for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:

Email: ncicteppubs@mail.nih.gov

The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/ proprietary information.

13.6 Genomic Data Sharing Plan

We will adhere to the NIH data sharing policy for GWAS (Policy for Sharing of Data Obtained in NIH Supported or Conducted Genome- Wide Association Studies (GWAS) (http://grants.nih.gov/grants/guide/notice-files/NOT-OD-07-088.html).
We will de-identify all human genomic data that we submit to NIH-designated data repositories according to the standards set forth in the HHS Regulations for the Protection of Human Subjects to ensure that the identities of research subjects cannot be readily ascertained with the data. We will also strip the data of identifiers according to the Health Insurance Portability and Accountability Act (HIPAA) Privacy Rule. The de-identified data will be assigned random, unique codes, and the key to other study identifiers will be held by the institution.
We will register all studies with human genomic data that fall within the scope of the GDS Policy in dbGaP by the time that data cleaning and quality control measures begin. After registration in dbGaP, we will submit the data to Cancer Genomics Hub, NIH-designated data repository.

14. REFERENCES

Adams W.C., Y.H. Chen., R. Kratchmarov, *et al.* (2016). Anabolism-Associated Mitochondrial Stasis Driving Lymphocyte Differentiation over Self-Renewal. *Cell Rep*. 17:3142-3152.

Agarwal, S.K., A.H. Salem, A.V. Danilov, *et al*., (2017). Effect of ketoconazole, a strong CYP3A inhibitor, on the pharmacokinetics of venetoclax, a BCL-2 inhibitor, in patients with non-Hodgkin lymphoma. *Br J Clin Pharmacol*. 83:846-854.

American Cancer Society . Key statistics acute myeloid leukemia (AML). https://wwwcancerorg/cancer/acute-myeloid-leukemia/about/key-statisticshtml (accessed Jan 28, 2019). 2019.

Antony-Debre I., A. Paul, J. Leite, *et al.* (2017). Pharmacological inhibition of the transcription factor PU.1 in leukemia. *J Clin Invest*. 127:4297-4313.

Bhagat T.D., D. Von Ahrens, M. Dawlaty, *et al*. (2019). Lactate-mediated epigenetic reprogramming regulates formation of human pancreatic cancer-associated fibroblasts. *Elife*. 8.

Bogenberger, J.M., D. Delman, N, Hansen, *et al.* (2015). Ex vivo activity of BCL-2 family inhibitors ABT-199 and ABT-737 combined with 5-azacytidine in myeloid malignancies. *Leuk Lymphoma.* 56:226-229.

Butler A., P. Hoffman, P. Smibert, et al. (2018). Integrating single-cell transcriptomic data across different conditions, technologies, and species. *Nat Biotechnol*. 36:411-420.

Caenepeel S., S.P. Brown, B. Belmontes, *et al.* (2018). AMG 176, a Selective MCL1 Inhibitor, Is Effective in Hematologic Cancer Models Alone and in Combination with Established Therapies. *Cancer Discov.* 8:1582-1597.

Cancer Genome Atlas Research Network, Ley TJ, Miller C, *et al*. (2013). Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N Engl J Med*. 368(22):2059-2074.

Carter C.C., A. Onafuwa-Nuga, L.A. McNamara, *et al.* (2010). HIV-1 infects multipotent progenitor cells causing cell death and establishing latent cellular reservoirs. *Nat Med*. 16:446-451.

Carter C.C., L.A. McNamara, A. Onafuwa-Nuga, *et al.* (2011). HIV-1 utilizes the CXCR4 chemokine receptor to infect multipotent hematopoietic stem and progenitor cells. *Cell Host Microbe*. 9:223-234.

Carvajal L.A., D.B. Neriah, A. Senecal, *et al.* (2018). Dual inhibition of MDMX and MDM2 as a therapeutic strategy in leukemia. *Sci Transl Med*. 10:eaao3003.

Cashen A.F., G.J. Schiller, M.R. O'Donnell, et. al. (2010). Multicenter, phase 2 study of decitabine for the first-line treatment of older patients with acute myeloid leukemia. *J Clin Oncol*. 28:556-561.

Chang B.S., A.J. Minn, S.W. Muchmore, *et al.* (1997). Identification of a novel regulatory domain in Bcl-X(L) and Bcl-2. *EMBO J*. 16:968-977.

Chen C., D. Siegel, M. Gutierrez *et al.* (2018). Safety and efficacy of selinexor in relapsed or refractory multiple myeloma and Waldenstrom macroglobulinemia. *Blood*. 131:855-863.

Chen J., Y. Kao, D. Sun, *et al.* (2018) MDS progression to AML at the stem cell level. .*Nature medicine*. 25:103-110.

Chen J., Y.R. Kao, D. Sun, *et al.* (2019a). Myelodysplastic syndrome progression to acute myeloid leukemia at the stem cell level. *Nat Med*. 25:103-110.

Chen X., C. Glytsou, H. Zhou, *et al*. (2019b). Targeting mitochondrial structure sensitizes acute myeloid leukemia to Venetoclax Treatment. *Cancer Discov*. 9:890-909.

Chyla B, Daver N, Doyle K, et al. (2018). Genetic Biomarkers Of Sensitivity and Resistance to Venetoclax Monotherapy in Patients With Relapsed Acute Myeloid Leukemia. *Am J Hematol*. 93:E202-E205.

Craddock C., Quek L., Goardon N., *et al.* Azacitidine fails to eradicate leukemic stem/progenitor cell populations in patients with acute myeloid leukemia and myelodysplasia. *Leukemia*. 2013;27(5):1028-1036.

Daver N, T.L. Dumlao, F. Ravandi, *et al*. (2013). Effect of NPM1 and FLT3 mutations on the outcomes of elderly patients with acute myeloid leukemia receiving standard chemotherapy. *Clin Lymphoma Myeloma Leuk*. 13:435-440.

Davids M.S., A.W. Roberts, J.F. Seymour, *et al.*, (2017). Phase I First-in-human study of ventoclax in patients with relapsed or refractory non-hodgkin lymphoma. *J Clin Oncol.* 35:826-833.

Deming P.B., J.C Rathmell. (2006). Mitochondria, cell death, and B cell tolerance. *Curr Dir Autoimmun*. 9:95-119.

DiNardo C.D, K.W. Pratz, V. Pullarkat, *et al.* (2019). Venetoclax combined with decitabine or azacitidine in treatment-naive, elderly patients with acute myeloid leukemia. *Blood*. 133:7-17.

DiNardo C.D., C.R. Rausch, C. Benton, *et al.* (2018c). Clinical experience with the BCL2-inhibitor venetoclax in combination therapy for relapsed and refractory acute myeloid leukemia and related myeloid malignancies. *Am J Hematol*. 93:401-407.

DiNardo C.D., E.M. Stein, S. de Botton, *et al.* (2018a). Durable Remissions with Ivosidenib in

IDH1-Mutated Relapsed or Refractory AML. *N Engl J Med.* 378(25):2386-2398.

DiNardo C.D., K.W. Pratz, A. Letai, *et al.* (2018b). Safety and preliminary efficacy of venetoclax with decitabine or azacitidine in elderly patients with previously untreated acute myeloid leukaemia: a non-randomised, open-label, phase 1b study. *Lancet Oncol*. 19:216-228.

Dinardo C.D., I.S. Tiong, A. Quaglieri, *et al*. (2020). Molecular patterns of response and treatment failure after frontline venetoclax combinations in older patients with AML. *Blood*. 2020;135:791-803.

Döhner H., E.H. Estey, D. Grimwade, *et al.* (2017). Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood*. 129:424-447.

Döhner H., E.H. Estey, S. Amadori, *et al.* (2010). Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. *Blood*. 115:453-474.

Dombret H., J.F. Seymour, A. Butrym, *et al.* (2015). International phase 3 study of azacitidine vs conventional care regimens in older patients with newly diagnosed AML with >30% blasts. *Blood*. 126:291-299.

Elias H.K., C. Schinke, S. Bhattacharyya, *et al.* (2014). Stem cell origin of myelodysplastic syndromes. *Oncogene*. 33:5139-5150.

Escudero S., E. Zaganjor, S. Lee, *et al.* (2018). Dynamic regulation of long-chain fatty acid oxidation by a noncanonicalinteraction between the MCL-1 BH3 Helix and VLCAD. *Mol Cell*. 69:729-743 e727.

Farge T., E. Saland, F. de Toni, *et al.* (2017). Chemotherapy-resistant human acute myeloid leukemia cells are not enriched for leukemic stem cells but require oxidative metabolism. *Cancer Discov*. 7:716-735.

Fernandez H.F., Z. Sun, X. Yao, *et al.* (2009). Anthracycline dose intensification in acute myeloid leukemia. *N Engl J Med.* 361:1249-1259.

Fesik S.W. (2001). BCL-2 AND IAP Proteins As Potential Drug Targets . *ScientificWorldJournal*. 1:105.

Fine JP, Gray RJ. (1999). A proportional hazards model for the subdistribution of a competing risk. *Journal of the American Statistical Association*. 94:496-509.

Fischer M.A., S.Y. Friedlander, M.P. Arrate, *et al.* (2020). Venetoclax response is enhanced by selective inhibitor of nuclear export compounds in hematologic malignancies. *Blood Adv.* 4:586-598.

Freise, K.J., B. Hu, and A.H. Salem. (2018). Impact of ritonavir dose and schedule on CYP3A4 inhibition and venetoclax clinical pharmacokinetics. *Eur J Clin Pharmacol*. 74:413-421.

Garcia-Manero G., E.A. Griffiths, D.P. Steensma, *et al.* (2020). Oral cedazuridine/decitabine for MDS and CMML: a phase 2 pharmacokinetic/pharmacodynamic randomized crossover study. *Blood*. 136:674-683.

Garcia-Manero G., S.D. Gore, C. Cogle, *et al.* (2011). Phase I study of oral azacitidine in myelodysplastic syndromes, chronic myelomonocytic leukemia, and acute myeloid leukemia. *J Clin Oncol*. 29(18):2521-2527.

Garcia-Manero G., J. McCloskey, E.A. Griffiths, *et al*., (2019). Pharmacokinetic exposure equivalence and preliminary efficacy and safety from a randomized cross over phase 3 study (ASCERTAIN study) of an oral hypomethylating agent ASTX727 (cedazuridine/decitabine) compared to IV decitatbine. *Blood*. 134(Supplement_1):846

Garzon R., M. Savona, R. Baz, *et al.* (2017). A phase 1 clinical trial of single-agent selinexor in acute myeloid leukemia. *Blood*. 129:3165-3174.

Gentles A.J., S.K. Plevritis, R. Majeti *et al.* (2010). Association of a leukemic stem cell gene expression signature with clinical outcomes in acute myeloid leukemia. *JAMA*. 304:2706-2715.

Gerriets V.A., R.J. Kishton, M.O. Johnson., *et al.* (2016). Foxp3 and Toll-like receptor signaling balance Treg cell anabolic metabolism for suppression. *Nat Immunol*. 17:1459-1466.

Glaser G., S.J. Weroha, M.A Becker., *et al.* (2015). Conventional chemotherapy and oncogenic pathway targeting in ovarian carcinosarcoma using a patient-derived tumorgraft. *PLoS One*. 10:e0126867.

Grimwade D., R.K. Hills, A.V. Moorman, *et al.* (2010). Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. *Blood*. 116:354-365.

Groeger J.S., A.B., Lucas H.T Thaler., *et al.* (1993). Infectious morbidity associated with long-term use of venous access devices in patients with cancer. *Ann Intern Med*. 119:1168-1174.

Haghverdi L, Lun ATL, Morgan MD, et al. (2018). Batch effects in single-cell RNA-sequencing data are corrected by matching mutual nearest neighbors. *Nat Biotechnol*. 36:421-427.

Hidalgo M, E. Bruckheimer, N.V. Rajeshkumar, *et al.* (2011). A pilot clinical study of treatment guided by personalized tumorgrafts in patients with advanced cancer. *Mol Cancer Ther*. 10:1311-1316.

Hidalgo M., F. Amant, A.V. Biankin., *et al.* (2014). Patient-derived xenograft models: an emerging platform for translational cancer research. *Cancer Discov*. 4:998-1013.

Horing E., A. Montraveta, S. Heine S, *et al.* (2017). Dual targeting of MCL1 and NOXA as effective strategy for treatment of mantle cell lymphoma. *Br J Haematol*. 177:557-561.

Investigator's Brochure. (2020). Venetoclax. AbbVie, Inc. North Chicago, IL. Edition 12, January 14, 2019.

Ishitobi M., K., Shibuya Y., Komoike *et al.* (2013). Preferences for oral versus intravenous adjuvant chemotherapy among early breast cancer patients. *Patient Prefer Adherence*. 7:1201-1206.

Jan M., Snyder T.M., M.R. Corces-Zimmerman, *et al.* (2012). Clonal evolution of preleukemic hematopoietic stem cells precedes human acute myeloid leukemia. *Sci Transl Med*. 4:149ra118.

Jilg S., V. Reidel, C. Muller-Thomas, *et al.* (2016). Blockade of BCL-2 proteins efficiently induces apoptosis in progenitor cells of high-risk myelodysplastic syndromes patients. *Leukemia*. 30:112-123.

Johnson M.O., M.M. Wolf, M.Z. Madden., *et al.* (2018). Distinct regulation of Th17 and Th1 cell differentiation by glutaminase-dependent metabolism. *Cell*. 175:1780-1795 e1719.

Kirimura S., M. Kurata M., Y. Nakagawa, *et al.* (2016). Role of microRNA-29b in myelodysplastic syndromes during transformation to overt leukaemia. *Pathology*. 48:233-241.

Kishton R.J., C.E. Barnes, A.G. Nichols., *et al.* (2016). AMPK is essential to balance glycolysis and mitochondrial metabolism to control T-ALL cell stress and survival. *Cell Metab*. 23:649-662.

Konopleva, M., D.A. Pollyea, J. Potluri, *et al.* (2016). Efficacy and biological correlates of response in a phase II study of venetoclax monotherapy in patients with acute myelogenous leukemia. *Cancer Discov*. 26:1106-1117.

Konopleva, M., R. Contractor, T. Tsao, *et al.* (2006). Mechanisms of apoptosis sensitivity and resistance to the BH3 mimetic ABT-737 in acute myeloid leukemia. *Cancer Cell*. 10:375-388.

Kotschy A, Szlavik Z, Murray J, *et al.* The MCL1 inhibitor S63845 is tolerable and effective in diverse cancer models. *Nature*. 2016;538(7626):477-482.

Kuderer N.M., D.C. Dale, J. Crawford J, *et al.*, (2006). Mortality, morbidity, and cost associated with febrile neutropenia in adult cancer patients. *Cancer*. 106:2258-2266.

Kuruvilla J., M. Savona, R. Baz, *et al.* (2017). Selective inhibition of nuclear export with selinexor in patients with non-Hodgkin lymphoma. *Blood*. 129:3175-3183.

Kuusanmaki H., A.M. Leppa, P. Polonen P, *et al.* (2020). Phenotype-based drug screening reveals association between venetoclax response and differentiation stage in acute myeloid leukemia. *Haematologica*. 105:708-720.

Lagadinou E.D, A. Sach, K. Callahan, *et al.* (2013). BCL-2 inhibition targets oxidative phosphorylation and selectively eradicates quiescent human leukemia stem cells. *Cell Stem Cell*. 12:329-341.

Lagadinou, E.D., A. Sach, K. Callahan, *et al.* (2013). BCL-2 inhibition targets oxidative phosphorylation and selectively eradicates quiescent human leukemia stem cells. *Cell Stem Cell*. 12:329-341.

Laille E., M.R. Savona, B.L. Scott, *et al.* (2014). Pharmacokinetics of different formulations of oral azacitidine (CC-486) and the effect of food and modified gastric pH on pharmacokinetics in subjects with hematologic malignancies. *J Clin Pharmacol*. 54:630-639.

Lancet J.E., G.L. Uy, J.E. Cortes, *et al.* (2018). CPX-351 (cytarabine and daunorubicin) liposome for injection versus conventional cytarabine plus daunorubicin in older patients with newly diagnosed secondary acute myeloid leukemia. *J Clin Oncol.* 36:2684-2692.

Lara-Astiaso D., A. Weiner, E. Lorenzo-Vivas, *et al.* (2014). Immunogenetics. Chromatin state dynamics during blood formation. *Science*. 345:943-949.

Larochelle A., M. Savona, M. Wiggins, *et al.* (2011). Human and rhesus macaque hematopoietic stem cells cannot be purified based only on SLAM family markers. *Blood*. 117:1550-1554.

Lee K.M., J.M. Giltnane, J.M. Balko, *et al.* (2017). MYC and MCL1 cooperatively promote chemotherapy-resistant breast cancer stem cells via regulation of mitochondrial oxidative phosphorylation. *Cell Metab*. 26:633-647 e637.

Lin K.H., P.S. Winter, A. Xie, *et al.* (2016). Targeting MCL-1/BCL-XL forestalls the acquisition of resistance to ABT-199 in acute myeloid leukemia. *Sci Rep*. 6:27696.

Lindsten T., A.J. Ross, A.King, *et al.* (2000). The combined functions of proapoptotic Bcl-2 family members bak and bax are essential for normal development of multiple tissues. *Mol Cell*. 6:1389-1399.

Liu G., Franssen E., Fitch M.I., *et al.* (1997). Patient preferences for oral versus intravenous palliative chemotherapy. *J Clin Oncol*. 15:110-115.

Liu Q., C.A. Herring, Q. Sheng, et al. (2018). Quantitative assessment of cell population diversity in single-cell landscapes. *PLoS Biol*. 16:e2006687.

Lo-Coco F., G. Avvisati, M. Vignetti, *et al.* (2013). Retinoic acid and arsenic trioxide for acute promyelocytic leukemia. *N Engl J Med.* 111-121.

Mahfouz R.Z., A. Jankowska, Q. Ebrahem, *et al.* (2013). Increased CDA expression/activity in males contributes to decreased cytidine analog half-life and likely contributes to worse outcomes

with 5-azacytidine or decitabine therapy. *Clin Cancer Res*. 19:938-948.

Mei M., I. Aldoss, G. Marcucci, V. Pullarkat, *et al.* (2019). Hypomethylating agents in combination with venetoclax for acute myeloid leukemia: Update on clinical trial data and practical considerations for use. *Am J Hematol*. 94:358-362.

Mitchell K., L. Barreyro, T.I. Todorova, *et al*. (2018) IL1RAP potentiates multiple oncogenic signaling pathways in AML. *J Exp Med*. 215:1709-1727.

Molokie R., D. Lavelle, M. Gowhari , *et al.* (2017). Oral tetrahydrouridine and decitabine for non-cytotoxic epigenetic gene regulationn in sickle cell disease: A randomized phase 1 study. *PLoS Med*. 14:e1002382.

Nabinger S.C., S. Chen, R. Gao, et al. (2019). Mutant p53 enhances leukemia-initiating cell self-renewal to promote leukemia development. *Leukemia*. 33:1535-1539.

Nechiporuk T., S.E.Kurtz, O. Nikolova, *et al.* (2019). The TP53 Apoptotic Network Is a Primary Mediator of Resistance to BCL2 Inhibition in AML Cells. *Cancer Discov*. 9:910-925.

Ng S.W., A. Mitchell, J.A. Kennedy, *et al.* (2016) A 17-gene stemness score for rapid determination of risk in acute leukaemia. *Nature*. 540:433-437.

Novershtern N., A. Subramanian , L.N. Lawton, *et al.* (2011). Densely interconnected transcriptional circuits control cell states in human hematopoiesis. *Cell*. 144:296-309.

Oltersdorf T., S.W.Elmore, A.R.Shoemaker, *et al.* (2005). An inhibitor of Bcl-2 family proteins induces regression of solid tumours. *Nature*. 435:677-681.

Owattanapanich W, Owattanapanich N, Kungwankiattichai S, *et al.* (2018). Efficacy and Toxicity of Idarubicin Versus High-dose Daunorubicin for Induction Chemotherapy in Adult Acute Myeloid Leukemia: A Systematic Review and Meta-analysis. *Clin Lymphoma Myeloma Leuk*. 18:814-821 e813.

Pang W.W., J.V.Pluvinage, E.A.Price, *et al.* (2013). Hematopoietic stem cell and progenitor cell mechanisms in myelodysplastic syndromes. *Proc Natl Acad Sci U S A*. 110:3011-3016.

Pautas C., F. Merabet, X. Thomas, *et al.* (2010). Randomized study of intensified anthracycline doses for induction and recombinant interleukin-2 for maintenance in patients with acute myeloid leukemia age 50 to 70 years: results of the ALFA-9801 study. *J Clin Oncol*. 28:808-814.

Pei S., D.A. Pollyea, A. Gustafson, *et al.* (2020). Monocytic subdones confer resistance to venetoclax-based therapy in patients with acute myeloid leukemia. *Cancer Discov*. 10:536-551.
Perl A.E., J.K. Altman, J. Cortes, *et al.* (2017). Selective inhibition of FLT3 by gilteritinib in relapsed or refractory acute myeloid leukaemia: a multicentre, first-in-human, open-label, phase 1-2 study. *Lancet Oncol*. 18:1061-1075.

Petros A.M., A. Medek, D.G.Nettesheim, *et al.* (2001). Solution structure of the antiapoptotic protein bcl-2. *Proc Natl Acad Sci U S A*. 98:3012-3017.

Pollyea D.A., B.M. Stevens, C.L. Jones, *et al.* (2018) Venetoclax with azacitidine disrupts energy metabolism and targets leukemia stem cells in patients with acute myeloid leukemia. *Nat Med*. 24:1859-1866.

Prata P.H., C. Bally, T. Prebet, *et al*. (2018). NPM1 mutation is not associated with prolonged complete remission in acute myeloid leukemia patients treated with hypomethylating agents. *Haematologica*. 103:e455-e457.

Ram R., O. Amit., T. Zuckerman, *et al.* (2019). Venetoclax in patients with acute myeloid leukemia refractory to hypomethylating agents-a multicenter historical prospective study. *Ann Hematol*. 98:1927-1932.

Ramsey H.E., M.A. Fischer, T. Lee, *et al.* (2018) A Novel MCL1 inhibitor combined with venetoclax rescues venetoclax-resistant acute myelogenous leukemia. *Cancer Discov*. 2018;8(12):1566-1581.

H. E. Ramsey, D. L Greenwood, S. Zhang, *et al*. (in press). Savona. BET inhibition enhances the antileukemic activity of low-dose Venetoclax in acute myeloid leukemia. *Clinical Cancer Research*

Ramsey HE, A. Oganesian, A.E. Gorska, *et al*. (2020). Oral azacitidine and cedazuridine approximate parenteral azacitidine efficacy in murine model. *Target Oncol.* 15:231-240.

Rathmell J.C., M.G. Vander Heiden, M.H. Harris. (2000). In the absence of extrinsic signals, nutrient utilization by lymphocytes is insufficient to maintain either cell size or viability. *Mol Cell*. 6:683-692.

Rego E.M., D. Ruggero, C. Tribioli, *et al.* (2006). Leukemia with distinct phenotypes in transgenic mice expressing PML/RAR alpha, PLZF/RAR alpha or NPM/RAR alpha. *Oncogene*. 25:1974-1979.

Rego E.M., L.Z. He, R.P. Warrell, Jr., *et al.* (2000). Retinoic acid (RA) and As2O3 treatment in transgenic models of acute promyelocytic leukemia (APL) unravel the distinct nature of the leukemogenic process induced by the PML-RARalpha and PLZF-RARalpha oncoproteins. *Proc Natl Acad Sci U S A*. 97:10173-10178.

Reyna D.E., T.P. Garner, A. Lopez, *et al.* (2017) Direct Activation of BAX by BTSA1 Overcomes Apoptosis Resistance in Acute Myeloid Leukemia. *Cancer Cell*. 32:490-505.e10.

Rowe J.M., H.T. Kim, P.A. Cassileth, *et al.* Adult patients with acute myeloid leukemia who achieve complete remission after 1 or 2 cycles of induction have a similar prognosis: a report on

1980 patients registered to 6 studies conducted by the Eastern Cooperative Oncology Group. *Cancer*. 116:5012-5021.

Savona M.R., D.A. Pollyea, W. Stock, *et al.* (2018a). Phase Ib Study of Glasdegib, a Hedgehog Pathway Inhibitor, in Combination with Standard Chemotherapy in Patients with AML or High-Risk MDS. *Clin Cancer Res.* 24:2294-2303.

Savona M.R., K. Kolibaba, P. Conkling P, *et al.* (2018b). Extended dosing with CC-486 (oral azacitidine) in patients with myeloid malignancies. *Am J Hematol*. 93:1199-1206.

Savona M.R., O. Odenike, P.C. Amrein, *et al.* (2019) An oral fixed-dose combination of decitabine and cedazuridine in myelodysplastic syndromes: a multicentre, open-label, dose-escalation, phase 1 study. *Lancet Haematol*. 6:e194-e203.

Schinke C., O. Giricz, W. Li, *et al*. (2015). IL8-CXCR2 pathway inhibition as a therapeutic strategy against MDS and AML stem cells. *Blood*. 125:3144-3152.

Schulte M.L., A. Fu, P. Zhao., *et al.* (2018). Pharmacological blockade of ASCT2-dependent glutamine transport leads to antitumor efficacy in preclinical models. *Nat Med*. 24:194-202.

Scott C.L., M.A. Becker, P. Haluska P. (2013). Patient-derived xenograft models to improve targeted therapy in epithelial ovarian cancer treatment. *Front Oncol*. 3:295.

Shastri A, Choudhary G, Teixeira M, *et al*. (2018). Antisense STAT3 inhibitor decreases viability of myelodysplastic and leukemic stem cells. *J Clin Invest*. 128(12):5479-5488.

Shaw S., Z. Bian, B. Zhao, *et al.* (2018). Optimization of Potent and Selective Tricyclic Indole Diazepinone Myeloid Cell Leukemia-1 Inhibitors Using Structure-Based Design. *J Med Chem*. 61:2410-2421.

Shenoy N., T.D. Bhagat, J. Cheville, *et al.* (2019). Ascorbic acid-induced TET activation mitigates adverse hydroxymethylcytosine loss in renal cell carcinoma. *J Clin Invest*. 2019;130:1612-1625.

Shlush L.I., S. Zandi, A. Mitchell, *et al.* (2014). Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. *Nature*. 506:328-333.

Siska P.J., G.J.van der Windt, R.J. Kishton, *et al.* (2016). Suppression of Glut1 and Glucose Metabolism by Decreased Akt/mTORC1 Signaling Drives T Cell Impairment in B Cell Leukemia. *J Immunol*. 197:2532-2540.

Siska P.J., K.E Beckermann., F.M. Mason, *et al.* (2017). Mitochondrial dysregulation and glycolytic insufficiency functionally impair CD8 T cells infiltrating human renal cell carcinoma. *JCI Insight*. 2:e93411.

Stein E.M., C.D. DiNardo, D.A. Pollyea, *et al.* (2017). Enasidenib in mutant IDH2 relapsed or

refractory acute myeloid leukemia. *Blood*. 2017;130(6):722-731.

Stevens B.M., C. L. Jones, A. Winters, *et al.* (2018). PTPN11 Mutations Confer Unique Metabolic Properties and Increase Resistance to Venetoclax and Azacitidine in Acute Myelogenous Leukemia. *Blood*. 132:909.

Stone R.M., R.A. Larson, H. Dohner. (2017). Midostaurin in FLT3-Mutated Acute Myeloid Leukemia. *N Engl J Med*. 2017;377:1903.

Swords R.T., S. Coutre, M.B. Maris, *et al.* (2018). Pevonedistat, a first-in-class NEDD8-activating enzyme inhibitor, combined with azacitidine in patients with AML. *Blood*. 131:1415-1424.

Teh T.C., N.Y. Nguyen, D.M. Moujalled, *et al.* (2018). Enhancing venetoclax activity in acute myeloid leukemia by co-targeting MCL1. *Leukemia*. 32:303-312.

Trapnell C., D. Cacchiarelli, J. Grimsby, *et al*. (2014). The dynamics and regulators of cell fate decisions are revealed by pseudotemporal ordering of single cells. *Nat Biotechnol*. 32:381-386.

Tsao, T., Y Shi., S. Kornblau, *et al.* (2012). Concomitant inhibition of DNA methyltransferase and BCL-2 protein function synergistically induce mitochondrial apoptosis in acute myelogenous leukemia cells. *Ann Hematol*. 91:1861-70.

Toldo S., R.W. Goehe, M. Lotrionte, *et al*. (2013). Comparative cardiac toxicity of anthracyclines in vitro and in vivo in the mouse. *PLoS One*. 8:e58421.

Vo, T.T., J. Ryan, R. Carrasco *et al.* (2012). Relative mitochondrial priming of myeloblasts and normal HSCs determines chemotherapeutic success in AML. *Cell*. 151:344-355.

Wei A.H., S.A. Strickland, J.Z. Hou J, *et al.* (2019). Venetoclax combined with low-dose venetoclax for previously untreated patients with acute myeloid leukemia: Results from a phase Ib/II study. *Journal of Clinical Oncology*. 37:1277-1284.

Wieand S, Schroeder G, O'Fallon JR. (1994). Stopping when the experimental regimen does not appear to help. *Stat Med.* 13:1453-1458.

Weroha S.J., M.A. Becker, S. Enderica-Gonzalez, *et al.* (2014). Tumorgrafts as in vivo surrogates for women with ovarian cancer. *Clin Cancer Res*. 20:1288-1297.

Will B., L. Zhou, T.O. Vogler, *et al.* (2012) Stem and progenitor cells in myelodysplastic syndromes show aberrant stage-specific expansion and harbor genetic and epigenetic alterations. *Blood*. 120:2076-2086.

Will B., T.O. Vogler, S. Narayanagari , *et al.* (2015). Minimal PU.1 reduction induces a preleukemic state and promotes development of acute myeloid leukemia. *Nat Med*. 21:1172-1181.

Woll P.S., Kjallquist U, Chowdhury O, *et al.* Myelodysplastic syndromes are propagated by rare and distinct human cancer stem cells in vivo. *Cancer Cell*. 25:794-808.

Wunderlich M., F.S. Chou, K.A. Link, *et al.* (2010). AML xenograft efficiency is significantly improved in NOD/SCID-IL2RG mice constitutively expressing human SCF, GM-CSF and IL-3. *Leukemia*. 24:1785-1788.

Wunderlich M., B. Mizukawa, F.S. Chou, *et al*. (2013). AML cells are differentially sensitive to chemotherapy treatment in a human xenograft model. *Blood*. 121:e90-97.

Yang A.S., M.R. Estecio, K.Doshi, *et al.* (2004). A simple method for estimating global DNA methylation using bisulfite PCR of repetitive DNA elements. *Nucleic Acids Res*. 32:e38.

Yates J.W., H.J. Wallace Jr., Jr., R.R. Ellison, *et al.* (1973). Cytosine arabinoside (NSC-63878) and daunorubicin (NSC-83142) therapy in acute nonlymphocytic leukemia. *Cancer Chemother Rep*. 57:485-488.

Zhao B., A.L. Arnold, M.A.Coronel, *et al.* (2018). Understanding the Species Selectivity of Myeloid Cell Leukemia-1 (Mcl-1) Inhibitors... *Biochemistry*. 57:4952-4958.

APPENDIX A 2017 ELN RECOMMENDATIONS: AML RISK

(Döhner *et al*., 2017)

2017 ELN risk stratification by genetics

Risk category[*]	Genetic abnormality
Favorable	t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*
	inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
	Mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low}[†]
	Biallelic mutated *CEBPA*
Intermediate	Mutated *NPM1* and *FLT3*-ITD^{high}[†]
	Wild-type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low}[†] (without adverse-risk genetic lesions)
	t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*[‡]
	Cytogenetic abnormalities not classified as favorable or adverse
Adverse	t(6;9)(p23;q34.1); *DEK-NUP214*
	t(v;11q23.3); *KMT2A* rearranged
	t(9;22)(q34.1;q11.2); *BCR-ABL1*
	inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2,MECOM(EVI1)*
	−5 or del(5q); −7; −17/abn(17p)
	Complex karyotype,[§] monosomal karyotype[‖]
	Wild-type *NPM1* and *FLT3*-ITD^{high}[†]
	Mutated *RUNX1*[¶]
	Mutated *ASXL1*[¶]
	Mutated *TP53*[#]

APPENDIX B PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX C FORMULA TO ESTIMATE RENAL FUNCTION USING SERUM CREATININE

Formulas to estimate renal function using serum creatinine provided by the NCI's Investigational Drug Steering Committee (IDSC) Pharmacological Task Force in table below.

1. Estimated glomerular filtration rate (eGFR) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) (Levey *et al.*, 2009).

Formulae:

Race and Sex	Serum Creatinine (SCr), *µmol/L (mg/dL)*	Equation
Black		
Female	≤62 (≤0.7)	$GFR = 166 \times (SCr/0.7)^{-0.329} \times (0.993)^{Age}$
	>62 (>0.7)	$GFR = 166 \times (SCr/0.7)^{-1.209} \times (0.993)^{Age}$
Male	≤80 (≤0.9)	$GFR = 163 \times (SCr/0.9)^{-0.411} \times (0.993)^{Age}$
	>80 (>0.9)	$GFR = 163 \times (SCr/0.9)^{-1.209} \times (0.993)^{Age}$
White or other		
Female	≤62 (≤0.7)	$GFR = 144 \times (SCr/0.7)^{-0.329} \times (0.993)^{Age}$
	>62 (>0.7)	$GFR = 144 \times (SCr/0.7)^{-1.209} \times (0.993)^{Age}$
Male	≤80 (≤0.9)	$GFR = 141 \times (SCr/0.9)^{-0.411} \times (0.993)^{Age}$
	>80 (>0.9)	$GFR = 141 \times (SCr/0.9)^{-1.209} \times (0.993)^{Age}$

SCr in mg/dL; Output is in mL/min/1.73 m^2 and needs no further conversions.

2. eGFR using the Modification of Diet in Renal Disease (MDRD) Study (Levey *et al.*, 2006).

175 x $SCr^{-1.154} \times age^{-0.203} \times 0.742$ (if female) × 1.212 (if black)
Output is in mL/min/1.73 m^2 and needs no further conversions.

3. Estimated creatinine clearance (ClCr) by the Cockcroft-Gault (C-G) equation (Cockcroft and Gault, 1976).

$$CLcr\ (mL/min) = \frac{[140 - age\ (years)] \times weight\ (kg)}{72 \times serum\ creatinine\ (mg\ /\ dL)} \{\times 0.85\ for\ female\ patients\}$$

Followed by conversion to a value normalized to 1.73 m^2 with the patient's body surface area (BSA).

References

1. Levey, A.S., L.A. Stevens, C.H. Schmid, *et al.* (2009). A new equation to estimate glomerular filtration rate. *Ann Inter Med*. 150:604-612.
2. Levey, A.S., J. Coresh, T. Greene, *et al.* (2006). Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Intern Med*. 145:247-254.
3. Cockcroft, D.W. and M.H. Gault. (1976). Prediction of creatinine clearance from serum creatinine. *Nephron*. 16:31-41.

APPENDIX D: PATIENT CLINICAL TRIAL WALLET CARD

NIH NATIONAL CANCER INSTITUTE

CLINICAL TRIAL WALLET CARD

Show this card to all of your healthcare providers and keep it with you in case you go to the emergency room.

Patient Name:

Diagnosis:

Study Doctor:

Study Doctor Phone #:

NCI Trial #: 10417

Study Drug(S): Venetoclax
ASTX727
Cytarabine
Daunorubicin

For more information: 1-800-4-CANCER
cancer.gov | clinicaltrials.gov

APPENDIX E PATIENT MEDICATION DIARY

CTEP-assigned Protocol #_10417____

Local Protocol # _____

PATIENT'S MEDICATION DIARY – Venetoclax and ASTX 727 CYCLE 1

Today's date _____ **Agent**_____

Patient Name_____ (*initials acceptable)* **Patient Study ID**_____

INSTRUCTIONS TO THE PATIENT:

1. Your dose of **ASTX727** will be one tablet per day on (circle one) Days 1-5 of each cycle. Take the tablets whole. Do not chew or split the tablet.
2. Take ASTX727 on an empy stomach (in the morning before breakfast). Do not eat for at least 2 hours beforeand 2 hours after taking **ASTX727**. If dose is missed or skipped, do not retake the dose unless you are told to do so.
3. Your dose of **venetoclax** will be (circle one) 4, 2, 1 tablets per day.
4. You will take venetoclax tablet(s) every day continuously (Days 1-28 of each cycle)
5. On days when receiving ASTX727, take venetoclax with breakfast at least 2 hours following ASTX727. On days you are not taking ASTX727, take venetoclax with breakfast at the same time each day. You should swallow the tablets whole. Do not chew. If dose is missed or skipped, do not retake the dose unless you are told to do so.
6. If you have any comments or notice any side effects, please record them in the Comments column.
7. Please return the forms to your physician when you go for your next appointment.

Day	What time was ASTX727 dose taken?	ASTX727 100mg/35mg tablet	What time was Venetoclax dose taken?	Venetoclax # of 100mg tablets taken	Comments
1					
2					
3					
4					
5					
6	(-)	Do not take dose.			
7	(-)	Do not take dose.			
8		Do not take dose.			
9	(-)	Do not take dose.			
10	(-)	Do not take dose.			
11	(-)	Do not take dose.			
12	(-)	Do not take dose.			

13	(-)	Do not take dose.			
14	(-)	Do not take dose.			
15		Do not take dose.			
16	(-)	Do not take dose.			
17	(-)	Do not take dose.			
18	(-)	Do not take dose.			
19	(-)	Do not take dose.			
20	(-)	Do not take dose.			
21	(-)	Do not take dose.			
22	(-)	Do not take dose.			
23	(-)	Do not take dose.			
24	(-)	Do not take dose.			
25	(-)	Do not take dose.			
26	(-)	Do not take dose.			
27	(-)	Do not take dose.			
28	(-)	Do not take dose.			

Physician's Office will complete this section:

1. Date patient started protocol treatment
   _____
2. Date patient was removed from study
   _____
3. Patient's planned total daily dose
   _____

4. Total number of pills taken this month (each size)
   _____
5. Physician/Nurse/Data Manager's Signature
   _____

Patient's signature: _____

APPENDIX F PATIENT MEDICATION DIARY FOR ANTIEMETIC PROPHYLAXIS FOR PATIENTS RECEIVING VENETOCLAX AND ASTX727

CTEP-assigned Protocol #_10417____

Local Protocol # _____

PATIENT'S MEDICATION DIARY – ANTIEMETIC PROPHYLAXIS

Today's date _____ **Agent**_____

Patient Name_____ (*initials acceptable)* **Patient Study ID**_____

INSTRUCTIONS TO THE PATIENT:

1. Your doctor will tell you what drug(s) and dose(s) to take to manage nausea/vomiting.
2. If you have any comments or notice any side effects, please record them in the Comments column.
3. Please return the forms to your physician when you go for your next appointment.

Day	What time was dose taken?	Name of drug and dosage	Comments
1			
2			
3			
4			
5			

Physician's Office will complete this section:

1. Date patient started protocol treatment
   _____
2. Date patient was removed from study
   _____
3. Patient's planned total daily dose
   _____
4. Total number of pills taken this month (each size)
   _____
5. Physician/Nurse/Data Manager's Signature
   _____

Patient's signature: _____

APPENDIX G PATIENT MEDICATION DIARY FOR ANTIEMETIC PROPHYLAXIS FOR PATIENTS RECEIVING 7+3 CHEMOTHERAPY

CTEP-assigned Protocol #_10417____
Local Protocol # _____

PATIENT'S MEDICATION DIARY – ANTIEMETIC PROPHYLAXIS

Today's date _____ **Agent**_____

Patient Name_____ (*initials acceptable)* **Patient Study ID**_____

INSTRUCTIONS TO THE PATIENT:

1. Your doctor will tell you what drug(s) and dose(s) to take to manage nausea/vomiting.
2. If you have any comments or notice any side effects, please record them in the Comments column.
3. Please return the forms to your physician when you go for your next appointment.

Day	What time was dose taken?	Name of drug and dosage	Comments
1			
2			
3			

Physician's Office will complete this section:

1. Date patient started protocol treatment
   _____
2. Date patient was removed from study
   _____
3. Patient's planned total daily dose
   _____
4. Total number of pills taken this month (each size)
   _____
5. Physician/Nurse/Data Manager's Signature
   _____

Patient's signature: _____

APPENDIX H BONE MARROW SAMPLE PROCESSING AND CRYOPRERVATION PROTOCOL

Collection:

Collect bone marrow aspirate in an acid citrate dextrose (ACD- yellow top) 7mL collection tube. Bone marrow samples may be held for no longer than 24 hours at 4°C before processing and may be processed in batches.

Ficoll separation:

Reagents:
Phosphate-buffered saline (PBS)
Fetal Bovine serum (FBS)
SepMate™ PBMC isolation kit (If SepMate™ kits are unavailable, please confirm alternate separation procedure with the PI)

1. Ensure that sample, phosphate-buffered saline with 2% fetal bovine serum (PBS + 2% FBS), density gradient medium, and centrifuge are all at room temperature (15 - 25°C).
2. Add density gradient medium to the SepMate™ tube by carefully pipetting it through the central hole of the SepMate™ insert. Refer to Table 1 (below) for required volumes. The top of the density gradient medium will be above the insert. Note: Small bubbles may be present in the density gradient medium after pipetting. This will not affect performance.
3. Dilute bone marrow sample with an equal volume of PBS + 2% FBS. Mix gently. For example, dilute 5 mL of sample with 5 mL of PBS + 2% FBS.
4. Keeping the SepMate™ tube vertical, add the diluted sample by pipetting it down the side of the tube. The sample will mix with the density gradient medium above the insert. Note: The sample can be poured down the side of the tube. Take care not to pour the diluted sample directly through the central hole.
5. Centrifuge at 1200 x g for 10 minutes at room temperature, with the brake on. Note: For samples older than 24 hours, a centrifugation time of 20 minutes is recommended.
6. Pour off the top layer, which contains the enriched MNCs, into a new tube. Do not hold the SepMate™ tube in the inverted position for longer than 2 seconds. Note: Some red blood cells (RBCs) may be present on the surface of the SepMate™ insert after centrifugation. This will not affect performance.
7. Wash enriched MNCs with PBS + 2% FBS. Repeat wash. Note: Centrifuging at 300 x g for 8 minutes at room temperature, with the brake on, is recommended, but can follow institutional guidelines to this end.

Table 1. Sample and Density Gradient Medium Volumes

SEPMATE™ TUBE	INITIAL SAMPLE (mL)	DENSITY GRADIENT MEDIUM (mL)
15	0.5 - 4.0	4.5
15	> 4 - 5	3.5
50	4 - 17	15

Automated Cell Quantification

Reagents:
0.4%Trypan blue solution
RPMI
Automated cell counter and cassettes

1. Add 20µL of 0.4% Trypan blue solution to a clean microfuge tube
2. Add 20µL of cell suspension to 80uL of RPMI to create a 1:5 dilution and mix by pipetting
3. Add 20uL of 1:5 dilution of cell suspension to the microfuge tube containing 20uL of 0.4% Trypan blue and mix by pipetting
4. Pipette 10uL into each side/counting chamber of a cassette
5. Slide cassette into cell counter
6. Press Zoom button and adjust focus of screen by using focus knob.
7. Press count cells and record the cell count and viability
8. If cell count exceeds limit of counter (>$1x10^7$cells/ml), make a 1:10 dilution of sample in RPMI media, then repeat counting procedure
9. Multiply viable cell count by the dilution factor to obtain true count of the recovered cells.

Cryopreservation Procedure

Reagents:
Fetal Bovine Serum (FBS)
DMSO
Cryovials
Mr. Frosty

1. Resuspend the remaining cell pellet in a freezing media ratio of 90% FBS and 10% DMSO with a minimum concentration of 5 million viable cells per mL.
2. Aliquot 1mL of cells into the provided cryovials. Each cryovial should contain a minimum of 5 million viable cells (maximum of 50 million viable cells per cryovial). For each prepared cryovial, please record the total number of viable cells per vial.
3. Place sample vials in Mr. Frosty container and place immediately in -80 Freezer.
4. Transfer samples into cryobox after 24 hours and store at -80 until shipment.
5. Batch ship samples on dry ice/frozen once a full cryobox is obtained via Fedex Priority overnight.

Bone Marrow Aspirate Smear Preparation

1. Write the participant's study number and date on the glass slide.
2. Place clean glass slide on a flat surface. Add one small drop of blood to one end.
3. One drop of aspirate material from bone marrow should be placed on the slide
4. Take another clean slide, and holding at an angle of about 45 deg, touch the blood with one end of the slide so the blood runs along the edge of the slide by capillary action.
5. Push carefully along the length of the first slide to produce a thin smear of blood with feathered edge as per local standard procedure.
6. Mount smears on slide with Cytoseal.
7. Please note that pathological data must be collected for the tubes processed to send to the Biobank

Preparation of Cytospin (Optional)

1. Pre-label positively charged glass slide with the participant's study number and date.
2. Prepare a cell suspension of $1x10^6$ cells/ml in PBS + 0.5% bovine serum albumin (BSA). Keep cells on ice.
3. Place the glass slide in the metal holder, then the CytoSep funnel with filter card, with the filter card facing the glass slide. Secure everything with clip.
4. Put the metal holder with the slide and funnel in the Slide Prep Plus machine.
5. Load 200ul of the cell suspension into the funnel. (If using a dual funnel, load 200ul in one side and 100ul in the other side)
6. Spin in Slide Prep Plus at 800rpm (55RCF) for 8min, Acceleration= Slow.
7. Carefully detach the funnel without smearing the fresh cytospin.
8. Dry slides 3hrs to overnight at room temperature, in the dark.

APPENDIX I Pharmacokinetic Study Sample Handling

1.1 Venetoclax Plasma Pharmacokinetic Samples

- The pharmacokinetic profile of venetoclax will be characterized by collecting blood samples (as outlined in the Study Calendar for Venetoclax+ASTX727 Combination and in the separate PK study uploaded at the Cancer Trials Support Unit website www.ctsu.org).
- Collection of Specimen(s): patients will have ~5 mL samples of blood collected in tubes containing K2 ETDA as an anticoagulant (purple top; e.g., Becton Dickinson Catalog # 367861 or 367844, Franklin Lakes, NJ) at the time points specified below:
    - **Course 1 Day 5** (Phase 1b and Phase 2 Venetoclax + ASTX727 Arm Only)
        - **Pre-venetoclax**
        - **1 hr post venetoclax**
        - **2 hr post venetoclax**
        - **4 hr post venetoclax**
        - **7 hr post venetoclax**
    - **Course 1 Day 22 (+/- 2 days)** (Phase 1b and Phase 2 Venetoclax + ASTX727 Arm Only)
        - **Pre-venetoclax**
        - **1 hr post venetoclax**
        - **2 hr post venetoclax**
        - **4 hr post venetoclax**
        - **7 hr post venetoclax**
- Obtain venous blood by standard phlebotomy technique from a peripheral access point. NOTE: Suggest using a minimum 18G needle to avoid sample hemolysis.
    - Obtain venous blood by standard phlebotomy technique from a peripheral access point.
- Fill-up the tubes as much as possible until blood flow stops.
- GENTLY invert each tube several times (8-10 times) immediately after collection to avoid sample hemolysis.
- Place samples immediately **on ice** after collection; samples must be processed **within 30 minutes**.

1.2 Handling of Specimens(s)

Processing instructions

1. Invert sample 8-10 times immediately before processing.
2. Centrifuge at ~1000-1300 x *g* (i.e., RCF, **not RPM**) for 10 minutes in swinging bucket (SW) or 15 minutes in a fixed angel (FA) rotor at 4ºC in a refrigerated centrifuge. Make sure that the centrifuge reaches speed and is maintained throughout the entire spin.
3. Carefully remove tube from centrifuge.
4. Using a pipette, transfer equal aliquots of plasma into 2-3 labeled 1.2 mL cryovials (e.g., preferred are external thread, conical self-standing vials like Corning™ 430658 or), not exceeding ~1 mL per cryovial.
5. Label samples as (drug) (matrix) (purpose), including protocol number (#10417), unique patient ID assigned at registration, date of collection, draw time, time point, protocol time point, and initials.
6. Store plasma samples at -70°C or below until shipment.

1.3 Shipping of Specimen(s)

Specimens should be stored through the end of Cycle 1 Day 22 and shipped as a batch by participant (more than one participant/shipment is acceptable if the site has >1 participant on-study). A participant's samples should be shipped to OSUCCC PhASR for ChOP-KC within 2 weeks of the last sample's collection date. (i.e., if C1D22 sample is collected on 9/1/2021, all of that participant's samples should be at the PhASR lab by 9/15/2021). OSUCCC PhASR may contact the study team to request shipment off-schedule.

Please ship 1 aliquots to the OSUCCC PhASR. Once receipt is confirmed, the back-up aliquot may be shipped. The back-up can be shipped at a later date with subsequent batches.

Preparing the shipment

*Samples should be stored in cardboard boxes (5 1/8" x 5 1/8" x 2", LxWxH) with dividers. (e.g., VWR Box item number is 82021-114; divider item number is 82007-154.)
*Please organize the samples by Patient and Time point in the box.
*Do not store in plastic bags (they break on dry-ice and labels will detach).
*A copy of each of the pharmacokinetic sample collection forms for the respective patients or a sample list should be included with each shipment. To prevent problems with illegible writing on tubes, consider numbering them (in addition to sample label) and numbering samples on the sample sheet.
*Note the study number, PI, and the drugs used/to be measured.
*A name, phone number and email address should be included with samples so that receipt can be acknowledged.
*Please notify the lab by email (phasr@osumc.edu) at least 24 hours prior to shipment.

Shipping

*All samples should be shipped via overnight express courier in insulated containers with enough dry ice to maintain the samples in a frozen state.
*Overnight shipments should occur on Monday through Wednesday (Tuesday is the preferred day) except when the following day is a holiday.

The OSUCCC PhASR
Attn: Kasey Hill, Ph.D. (Venetoclax Samples)
441 Biomedical Research Tower
460 West 12th Avenue
Columbus, OH 43210
Phone: (614) 688-0578
Fax: (614) 292-7766