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Title: Multicenter phase 1/2 study of combination therapy with the DNA methyltransferase inhibitor decitabine and the poly ADP ribose polymerase (PARP) inhibitor talazoparib (BMN 673) for untreated acute myeloid leukemia (AML) in adult patients unfit for cytotoxic chemotherapy or relapsed/refractory AML

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SCHEMA

Phase 1

Decitabine is administered intravenously (IV) daily for 5 days every 28 days through Cohort 6. Decitabine is administered intravenously (IV) daily for 10 days every 28 days in Cohort 7.

Talazoparib is administered orally daily on days 1-28.

		Talazoparib* (mg)			
		0.25	0.50	0.75	1.0
Decitabine* (mg/m^2)	10	1	1a	1b	1c
	15	2	2a	2b	2c
	20	3	4	5	6

***NOTE: DECITABINE IS DOSED IN MG/M^2, BUT TALAZOPARIB IS DOSED IN MG.**

The 'outer layer' of this nested dose escalation trial will escalate the dose of the two drugs by sequentially going through dose levels 1-6 in the table. The standard algorithm of the 3+3 design will be applied as specified below.

If the MTD has not been reached at dose level 6, the RP2D of decitabine will be 20 mg/m^2 combined with a dose of talazoparib of 1.0 mg. If 2 or more participants develop DLT at dose level 1, the combination of decitabine and talazoparib will be considered too toxic for further clinical development.

If the *provisional* MTD for decitabine in combination with talazoparib is dose level 1 or 2, one or the other of the 'inner layers' of the trial will be activated. If the provisional MTD corresponds to level 2, the dose of decitabine will be fixed at 15 mg/m^2 and the dose of talazoparib will be escalated through steps 2a-c, again using the algorithm of the 3+3 design below. If the tolerance is not exceeded at level 2c, then the RP2D of decitabine is 15 mg/m^2 combined with a dose of talazoparib of 1.0 mg.

Similarly, if the provisional MTD for the combination of the two agents from the outer layer is level 1, the dose of decitabine will be fixed at 10 mg/m^2 and the dose of talazoparib will be escalated through steps 1a-c, again using the algorithm of the 3+3 design below to define the MTD of the combination.

If Cohort 6, with 20 mg/m^2 decitabine daily for 5 days and talazoparib 1 mg daily, is completed without dose-limiting toxicity, Cohort 7, consisting of decitabine, 20 mg/m^2 daily for 10 days and talazoparib, 1 mg daily, will be added.

Following completion of Cohort 6 or Cohort 7 without dose-limiting toxicity, a minimum of 9 or maximum of 17 participants not previously treated with DNMTis (decitabine, azacitidine and/or guadecitabine) will be enrolled to assess response in this patient population.

Cohort 7 will consist of a minimum of 3 participants with a maximum of 6 participants.

Phase 2

Decitabine is administered intravenously (IV) daily for 5 or 10 days every 28 days, depending on Phase 1 results.

Talazoparib is administered orally daily days 1-28.

Phase 2 doses and schedule will be determined based on data from Phase 1.

Phase 2, Trial A – a minimum of 25 participants up to a maximum of 63 participants will be enrolled in the Trial A portion of Phase 2

Phase 2, Trial B – a minimum of 22 participants to a maximum of 40 participants will be enrolled in the Trial B portion of Phase 2

Total Phase 2 enrollment – a total minimum of 47 and a total maximum of 103 participants will be enrolled in Phase II of this study

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1. OBJECTIVES

1.1 Primary Objectives

1.1.1 Phase 1

To determine the recommended Phase 2 doses of combined decitabine intravenously (IV) daily for 5 or 10 days and talazoparib orally daily continuously in patient participants with refractory or relapsed acute myeloid leukemia (AML).

1.1.2 Phase 2

1.1.2.1 To determine the safety of decitabine and talazoparib combination therapy in newly diagnosed AML patient participants unfit for intensive chemotherapy.

1.1.2.2 To determine the efficacy of decitabine and talazoparib combination therapy in newly diagnosed AML patient participants unfit for intensive chemotherapy.

1.1.2.3 To determine the safety of decitabine and talazoparib combination therapy in patient participants with relapsed and refractory AML.

1.1.2.4 To determine the efficacy of decitabine and talazoparib combination therapy in patient participants with relapsed and refractory AML.

1.1.2.5 To explore the safety of decitabine and talazoparib therapy in AML patient participants previously treated with a DNA methyltransferase inhibitor (azacitidine, decitabine or guadecitabine) for AML or for antecedent MDS.

1.1.2.6 To explore the efficacy of decitabine and talazoparib therapy in AML patient participants previously treated with a DNA methyltransferase inhibitor (azacitidine, decitabine or guadecitabine) for AML or for antecedent MDS.

1.1.2.7 To determine treatment duration, disease-free survival (DFS) and overall survival (OS) for each of the three patient participant cohorts.

1.2 Secondary Objectives

1.2.1 Phase 1

1.2.1 To observe and record anti-tumor activity while on protocol therapy. Although the clinical benefit of this drug combination has not yet been established, the intent of offering this treatment is to provide a possible therapeutic benefit, and thus participants will be carefully monitored for tumor response and symptom relief in addition to safety and tolerability.

1.2.2 To explore pharmacodynamic effects of decitabine IV daily for 5 or 10 days every 28 days and talazoparib orally daily continuously in patient participants with relapsed or refractory AML.

1.2.3 To explore cytogenetic and molecular predictors of response to decitabine and talazoparib combination therapy.

1.2.2 Phase 2

1.2.2.1 To explore biomarker correlates of response.

1.2.2.2 To explore pharmacodynamic correlates of response.

2. BACKGROUND

2.1 Acute Myeloid Leukemia

AML is a malignancy that arises in a hematopoietic stem cell in the bone marrow and is characterized by maturation arrest of a malignant clone of myeloid cells (1,2). Immature myeloid cells, or blasts, accumulate in the bone marrow, with failure of normal hematopoiesis. AML is fatal if not effectively treated. The initial goal of treatment is induction of complete remission (CR), defined by normal blood counts and normal bone marrow morphology, or, less preferably, complete remission with incomplete count recovery (CRi), defined by normal bone marrow morphology but incomplete blood count recovery. Post-remission therapy is then administered with the goal of prolonging disease-free survival (DFS) and maximizing the likelihood of cure.

The mainstay of remission induction therapy for AML for the last 40 years has been combination chemotherapy including the nucleoside analogue cytarabine and an anthracycline, usually daunorubicin or idarubicin (1,2). Other chemotherapy drugs, including etoposide, mitoxantrone, topotecan, flavopiridol and the nucleoside analogs cladribine, clofarabine or fludarabine have been added or substituted in diverse treatment regimens. Post-remission therapy may consist of high-dose cytarabine or hematopoietic stem cell transplantation (HSCT). While the backbone of AML therapy has changed little, recognition of subsets of patients who are unlikely to achieve long-term DFS has allowed appropriate application of allogeneic HSCT, and enrollment on clinical trials when standard therapy is unlikely to be effective. Unfortunately, current 5-year survival for AML patients is only 23% (2). Survival has improved over several decades in younger adults, but not in those with prognostically unfavorable AML cell karyotypes, or with the prognostically unfavorable molecular abnormality fms-like tyrosine kinase 3 internal tandem duplication (FLT3-ITD). Other subgroups that fare poorly include older adults and those with AML evolved from an antecedent marrow disorder such as a MDS or with therapy-related AML (t-AML) (1,2). New therapies are needed, especially for patients in groups with poor responses to current cytotoxic chemotherapy. Patients with AML resistant to, or relapsing after, cytotoxic chemotherapy also need new approaches.

Untreated AML patients may be considered “unfit for chemotherapy” if deemed unlikely to tolerate chemotherapy or to respond to chemotherapy, based on patient and/or disease characteristics. Patient characteristics may include poor performance status, comorbidities and/or advanced age, while disease characteristics may include prognostically unfavorable AML karyotype and/or AML evolved from an antecedent marrow disorder such as MDS, or t-AML (1,2).

AML patients considered unfit for chemotherapy and patients with AML resistant to, or relapsing after, cytotoxic chemotherapy are currently often treated with DNA methyltransferase inhibitors (DNMTis). To improve responses, combinations of DNMTis and novel agents are being studied in clinical trials in these patients (1,2).

2.2 Decitabine

Decitabine, or 5-aza-2'-deoxycytidine is a cytidine analog that functions as a DNA methyltransferase inhibitor (DNMTi) and was found to induce cellular differentiation at low concentrations by reversing DNA methylation-induced gene silencing (3).

Clinical testing of decitabine was undertaken in MDS. Following promising data in initial small phase 2 trials in MDS (4), Wijermans et al. performed a large multicenter phase II trial of decitabine a dose of 15 mg/m^2 infused intravenously (IV) over a 4-hour period every 8 hours for 3 consecutive days, repeated every 6 weeks (5). The overall response rate was 49% for all patients, and was 64% in patients with a high-risk International Prognostic Scoring System (IPSS) score, and response duration was 36 weeks for patients who achieved CR. In the first phase III trial of decitabine (6), 170 patients with MDS, including 70% with higher-risk IPSS scores, were randomized to receive either decitabine in the above schedule or best supportive care (BSC). The overall improvement rate in the decitabine arm was 30%, compared with 7% in the BSC arm, and time to AML or death was significantly prolonged in patients with higher-risk MDS. Decitabine was FDA-approved for treatment of MDS with the above schedule on May 2, 2006.

There were subsequent efforts to optimize the decitabine schedule for treatment of MDS. Patients with higher-risk MDS and chronic myelomonocytic leukemia (CMML) were randomly assigned to one of three treatment schedules: 20 mg/m^2 IV daily for five days, 20 mg/m^2 subcutaneously (SQ) daily for five days, and 10 mg/m^2 IV daily for ten days. The five-day IV schedule produced a CR rate of 39%, compared with 21% and 24% in the five-day SQ and ten-day IV schedule, respectively, and was selected as optimal (7). Steensma et al. (8) studied the 5-day IV schedule, repeated every 4 weeks, in 99 MDS patients, demonstrating an overall response rate of 32% (17 CR and 15 marrow CRs), and an overall improvement rate of 51%, included 18% hematologic improvement (HI). The 5-day dosing regimen was approved for treatment of MDS on March 12, 2010.

Decitabine also has efficacy in AML and is well tolerated, and therefore is also currently in widespread use to treat AML in patients who are previously untreated and “unfit” for chemotherapy or who have refractory or relapsed AML with low likelihood of response to intensive chemotherapy (9-14). Cashen et al. (9) treated 55 older previously untreated AML patients with decitabine 20 mg/m^2 IV daily for 5 days every 4 weeks. Importantly, decitabine was continued until disease progression or an unacceptable adverse event occurred; patients received a median of three (range, one to 25) cycles. The overall response rate was 25% (CR rate, 24%). Overall median survival was 7.7 months, and 30-day mortality rate 7%. The most common toxicities were myelosuppression, neutropenic fever and fatigue. In a phase II, multicenter study of decitabine in the same schedule as first-line therapy in 227 older patients with AML (10), 59 patients (26%) achieved CR, including 30 with CR and 29 with PR, and 60 (26%) had a lesser antileukemic effect, for an overall response rate of 52%. Median overall survival was 5.5 months and one-year survival rate, 28%. Toxicities were predominantly hematologic.

In a subsequently reported multicenter, randomized, open-label, phase III trial, 485 older patients with newly diagnosed AML were randomly assigned 1:1 to 5-day decitabine or either supportive care or low-dose cytarabine (11). The primary end point was OS; the secondary end point was rate of CR plus CR without platelet recovery (CRp). The primary analysis with 396 deaths (81.6%) showed a non-significant increase in median OS with decitabine, 7.7 months (95% CI, 6.2 to 9.2) versus 5.0 months (5% CI, 4.3 to 6.3) with P = .108, hazard ratio [HR], 0.85; 95% CI, 0.69 to 1.04). An unplanned analysis with 446 deaths (92%) showed a survival benefit for decitabine (HR,

0.82; 95% CI, 0.68 to 0.99; nominal P = .037). The most common drug-related AEs with decitabine were thrombocytopenia (27%) and neutropenia (24%). Decitabine was approved for treatment of AML in Europe based on the results of this study. It was not approved for treatment of AML in the US, but is in widespread use off-label. As with decitabine treatment of MDS, decitabine treatment for AML continues on an ongoing basis, until disease progression.

Decitabine has also been used in a 10-day regimen for AML (12-14). The ten-day decitabine regimen produced response rates of 64% (47% CR and 17% CRi), 40.4% (CR) and 42% (31% CR and 11% CRi) in untreated AML patients unfit for chemotherapy (10-12). Survival in responders was 481 days (approximately 16 months) and 19.4 months in the two series in which it was stated (13,14). In contrast, the 10-day decitabine regimen produced a CR rate of only 15.7% in patients with refractory or relapsed AML (13).

2.3 Talazoparib

Talazoparib (BMN 673) (15) is an orally bioavailable inhibitor of the nuclear enzyme poly ADP ribose polymerase (PARP), with potential antineoplastic activity. Talazoparib selectively binds to PARP and prevents PARP-mediated DNA repair of single-strand DNA breaks via the base excision repair pathway. This enhances the accumulation of DNA strand breaks, promotes genomic instability and eventually leads to apoptosis. PARP catalyzes post-translational ADP-ribosylation of nuclear proteins that signal and recruit other proteins to repair damaged DNA and is activated by single-strand DNA breaks.

Talazoparib is a PARP inhibitor (PARPi) with superior potency (PARP1 IC_{50} 0.57 nmol/L) and favorable metabolic stability, oral bioavailability, and pharmacokinetic properties (15,16). In addition, talazoparib is able to trap PARP in the low nanomolar range (15).

in vitro metabolism studies of talazoparib in hepatic microsomes from rats, dogs and humans demonstrated that talazoparib has >90% stability over 2 hours in all three species. Fecal excretion was the major route of elimination.

It is unlikely that talazoparib will demonstrate CYP450 inhibition- or induction-based drug-drug interactions when co-administered with corresponding substrates. Treatment with up to 10 µM talazoparib in human liver microsomes showed no direct, time- or metabolism-dependent inhibition of CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6 or 3A4 enzymes.

Clinical trials of talazoparib to date in patients with solid tumors have demonstrated good tolerability and established a dose of 1 mg orally (PO) daily (17-19). The dose-limiting toxicity was thrombocytopenia (18).

A phase 1 clinical trial of talazoparib in advanced hematologic malignancies was initiated in 2011. This was a two-arm, open-label study to determine the MTD and assess the safety, pharmacokinetics, pharmacodynamics, and preliminary efficacy of talazoparib in patients with AML, MDS, chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL). Arm 1 enrolled patients with either AML or MDS. Arm 2 enrolled patients with either CLL or MCL. The study enrolled 33 patients and was closed in May 2014. Results were reported at the 2014 European Hematology Association (EHA) meeting (20). Oral talazoparib was administered on a continuous daily schedule (21-day cycle) at escalating dose levels. Thirty-three patients (Arm 1: 21 AML, 4 MDS; Arm 2: 4 CLL, 4 MCL), 10F/23M, median age 71 years (range 22-86), ECOG performance status 0-1, were enrolled. Median number of previous regimens was 3 (range, 1-7) in Arm 1 and

6 (range 1-13) in Arm 2. Talazoparib dose levels were: 100, 200, 300, 450, 900, 1350 and 2000 µg/day. Dose-limiting toxicities (DLTs) included neutropenic fever and neutropenic sepsis in 2/4 patients at 2000 µg/day in Arm 1 and severe neutropenia in 2/5 patients at 900 µg/day in Arm 2. Overall, the most frequent drug-related adverse events were fatigue (all grades=27%), neutropenia (27%), nausea (24%), infections (21%), and thrombocytopenia (12%). No alopecia was reported. No responses were seen. Stable disease was seen in 13/25 patients in Arm 1 and 5/8 patients in Arm 2. One MDS patient received 24 cycles of talazoparib (484 days) and became red blood cell (RBC) transfusion-independent. It was concluded that the MTD of talazoparib appeared to be different in patients with AML, MDS, CLL and MCL, and that talazoparib had limited activity as a single agent in a combined group of patients with advanced hematological malignancies.

In the novel combination regimen to be tested in this clinical trial, talazoparib will be studied at doses of 0.25, 0.5, 0.75 and 1 mg.

2.4 Rationale

2.4.1 DNA Methyltransferase Inhibitors

In addition to causing gene promoter hypomethylation and consequent gene re-expression, DNMTis also have additional mechanisms of action in MDS and AML. In particular, decitabine has been found to cause formation of DNA double-strand breaks (DSBs), with repair of this damage following drug removal (21). Decitabine also induces reactive oxygen species (ROS) in myeloid leukemia cells (22).

2.4.2 PARP-dependent Aberrant DNA Repair in AML

The Rassool laboratory has demonstrated upregulation of a highly error-prone alternative non-homologous end-joining (alt NHEJ) pathway for repair of DNA DSBs in myeloid leukemias, with increased expression of its component proteins, including PARP (23-25). PARP was initially characterized as a key component of base excision repair of DNA single-strand breaks, but was subsequently found to also be a key part of the alt NHEJ DNA DSB repair pathway, which functions as backup to the classical NHEJ pathway. The alt NHEJ DNA DSB repair pathway is sensitive to PARPis, which inhibit poly (ADP-ribose) synthesis by PARP-1 (26). Myeloid leukemia cells were shown to be sensitive to PARPis, suggesting their dependence on PARP for survival (27).

2.4.3 PARP Inhibitors

PARPis are in clinical trials in patients with homologous recombination-deficient tumors, primarily breast and ovarian cancers in patients with *BRCA* mutation. They are also being tested as radiosensitizers and chemosensitizers in sporadic cancers (28).

The PARPis most extensively studied to date include olaparib (AZD-2281) and veliparib (ABT-888) (28). Other PARPis include niraparib (MK-4827), CEP-9722, E7016/GPI-21016, rucaparib (AG-014699/PF-01367338) and talazoparib (28). It was recently shown that PARPis differ markedly in their ability to trap PARP-1-DNA complexes, with the extent of trapping correlating with cytotoxicity: niraparib (MK-4827)> olaparib (AZD-2281)>>veliparib (ABT888) (29). Talazoparib is able to trap PARP in the low nanomolar range (15) and has superior potency (PARP1 IC_{50} 0.57 nmol/L) (15).

There are relatively few data on PARPis in AML. The PARPi ABT-888 was found to sensitize myeloid leukemia cell lines to temozolamide (30), and the PARPi olaparib was recently found to disrupt base excision repair of lesions induced by decitabine (31). There is also little clinical experience in AML. Several clinical trials have been undertaken, with results not yet reported.

The PARPi to be studied in this clinical trial is the novel potent PARPi talazoparib (15).

2.4.4 Combined DNA Methyltransferase and PARP Inhibitors in AML

The Baylin laboratory demonstrated that transient exposure to DNMTis at low nM concentrations reprograms cancer cells, altering gene expression patterns in key cellular pathways, including DNA repair pathways (32). They and others have also demonstrated that DNMTs interact with PARP in complexes (33). Since DNMTis trap DNMT on DNA and chromatin, it was hypothesized that co-treatment of leukemia cells with DNMTis would further trap PARP in chromatin and at DSBs, sensitizing cells to PARPis. Testing this hypothesis has been a focus of the Rassool laboratory.

The Rassool laboratory has demonstrated that pre-treatment of myeloid leukemia cell lines and AML patient samples with decitabine at low nanomolar (20 nM) concentrations sensitizes them to PARPis, including ABT-888 and talazoparib (34). These combinations induce a significant decrease in methylcellulose colony formation, compared to treatment with DNMTi or PARPi alone. This was particularly evident in cell lines (2 of 4) and primary patient samples (3 of 6) with FLT3/ITD mutations, which are present in AML cells in approximately a third of patients and are associated with unfavorable prognosis. Moreover, decitabine and PARPis appear to cooperate to enhance the basic mechanism underlying the activity of PARPis, increasing the cytotoxic DNA-PARP complexes on chromatin, resulting in retention of PARP1 and DNMT1 at DSBs and decreased DNA DSB repair. Additionally, we have found that DNMTis decrease expression of homologous recombination (HR) DSB repair genes, suggesting that HR activity is also impaired through this mechanism. These data support combined DNMTi and PARPi as a novel treatment regimen for myeloid malignancies. This concept has now also been validated *in vivo* in immunodeficient mice engrafted with MV4-11 or MOLM14 cells, human leukemia cell lines with FLT3-ITD, treated simultaneously with the DNMTi azacitidine and the PARPi talazoparib. In this scenario, concurrent administration of azacitidine 0.5 mg/kg + talazoparib 0.1 mg/kg markedly suppresses tumor growth for at least 30 days, in relation to treatment with either drug alone (34).

Patients with MDS and AML respond to DNMTis, but response rates and response duration need to be improved, and combinations with other agents are being studied. DNMTis may sensitize cells to other therapeutic agents, and this sensitization may serve as a basis for novel combination regimens. Based on our preclinical data, we propose a novel regimen combining DNMTis and PARPis.

2.4.5 Clinical Trial Strategy

In this clinical trial, we combine decitabine and talazoparib in the treatment of patient participants with AML. Decitabine will be given in the established regimen of IV daily dosing for 5 or 10 days every 28 days. Talazoparib will be initiated orally daily on a continuous basis, beginning on Day 1 of Cycle 1. In this phase 1 trial, decitabine and talazoparib will be tested at 3 and 4 dose levels, respectively, yielding up to 6 combinations. Doses will be escalated based on tolerability using a 'classic' 3+3 design. Once the MTD for decitabine combined with 0.25 mg talazoparib is established, the dose of talazoparib will be escalated with the dose of decitabine kept fixed at the

provisional MTD dose. There is no further stepping up or down of the drugs in the combination.

The regimen for phase 2 testing will be chosen based on tolerability, but also based on available pharmacodynamic and efficacy data. In phase 2, the selected combination regimen will be studied for efficacy.

Each participant will be treated until occurrence of either unacceptable toxicity or disease progression. Bone marrow aspirate and biopsy will be performed on approximately day 28 of each treatment cycle unless circulating blasts persist in the peripheral blood, until documentation of CR or CRi. Bone marrow aspirate and biopsy will then be repeated at time of clinical concern for disease progression.

In Phase 1, the clinical trial will enroll patient participants with relapsed and refractory AML in order to test the novel combination initially in patients with less favorable outcomes with decitabine alone. This is being done to avoid the possibility of compromising the outcomes of untreated patients, who have up to a 40% response rate to 5-day decitabine as a single agent, in the unlikely possibility that the combination is inferior. The regimen will then also be tested in previously untreated AML patient participants unfit for chemotherapy in phase 2.

2.5 Correlative Studies

2.5.1 Pharmacodynamic Studies

Pharmacodynamic studies will be performed in cycle 1 on blood samples obtained pre-treatment on Day 1, and on Day 5 one hour after the decitabine injection, and on Day 8 anytime. These studies will be conducted on all participants. For participants receiving 10-day decitabine, sampling will be on Days 1, 5, 8 and 10, one hour after the decitabine injection.

As detailed in 2.4.4, our mechanistic hypothesis is that decitabine and talazoparib will cooperate to increase the cytotoxic DNA-PARP complexes on chromatin, resulting in decreased DNA DSB repair and decreased cell survival. The assays in 2.5.1.1 and 2.5.1.2 will test components of this mechanism.

2.5.1.1 Colony Formation, Immunofluorescence, PARP Trapping, mRNA and Protein Expression, and DNA DSB Repair.

1) Colony-forming assays
2) Immunostaining for γH2AX (a marker for DNA DSBs), RAD51 and other DSB repair, epigenetic and DNA replication markers.
3) PARP trapping
4) PAR assay
5) Protein expression of DSB repair genes, including PARP1, PAR, RAD51, KU70/80 and DNA ligase 3, and epigenetic markers, including EZH2 and DNMT1, by Western blot analysis
6) Levels of mRNA expression of DSB repair genes, including PARP1, RAD51, KU70/80 and DNA ligase 3, by quantitative polymerase chain reaction (Q-PCR)
7) Levels of DSB repair, measured using *in vitro* plasmid-based assays

These studies will be performed in the laboratory of Dr. Rassool at UMGCCC.

2.5.1.2 DNA methylation

Changes in DNA methylation will also be studied, as follows:

1) Whole genome methylation analyses using the Infinium 450K array platform.
2) Bisulfite-Pyrosequencing of Line 1 and 10 tumor suppressor genes.

Samples will be shipped to the Van Andel Research Institute (VARI), where they will be processed and distributed for the above studies. The studies will be performed in core laboratories of VARI-Stand Up to Cancer (SU2C) Epigenetics Dream Team members or collaborators.

2.5.1.3 Immune response endpoints

These studies will be performed in the future if funding allows. T-cell response pre- and post-treatment and cytokine release will be assessed.

2.5.2 Potential Predictors of Response

Potential predictors of response will be explored, including karyotype, *FLT3* and *NPM1* mutations, mutations in genes regulating methylation, including *DNMT3A*, *TET2*, *IDH1*, *IDH2* and *EZH2*, as well as *PARP* expression levels.

These studies will be performed on pre-treatment bone marrow, or on blood if marrow is inaspirable. Karyotype will be determined by standard cytogenetic analysis at each institution. Mutations will be studied as part a myeloid gene mutation panel. *PARP* expression levels and PAR activity will be measured in Dr. Rassool's laboratory as part of the studies detailed above. Low baseline levels of homologous recombination DSB repair activity may also predict response to the drug combination and will also be explored.

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria

1) Diagnosis of AML based on 2008 WHO criteria (35). AML may be de novo, following a prior hematologic disorder, including MDS or Philadelphia chromosome-negative myeloproliferative neoplasm, and/or therapy-related.

2) **PHASES 1 AND 2:** Patient participants with AML that has relapsed after, or is refractory to, first-line therapy, with or without subsequent additional therapy, and are currently considered unfit for, or unlikely to respond to, cytotoxic chemotherapy.

3) **PHASE 2 ONLY:** Patient participants previously untreated for AML who are considered unfit for cytotoxic chemotherapy by virtue of performance status, comorbidities, advanced age and/or low likelihood of response based on disease characteristics, or who decline cytotoxic induction chemotherapy.

4) Patient participants who have undergone autologous stem cell transplantation (autoSCT) are eligible provided that they are $\geq$ 4 weeks from stem cell infusion.

5) Patient participants who have undergone allogeneic SCT (alloSCT) are eligible if they are ≥ 60 days from stem cell infusion, have no evidence of graft versus host disease (GVHD) > Grade 1, and are ≥ 2 weeks off all immunosuppressive therapy.

6) Previous cytotoxic chemotherapy must have been completed at least 3 weeks and radiotherapy at least 2 weeks prior to Day 1 of treatment on the study, and all adverse events (excluding alopecia) due to agents administered more than 4 weeks earlier should have recovered to < Grade 1. Participants with hematologic malignancies are expected to have hematologic abnormalities at study entry. Hematologic abnormalities that are thought to be primarily related to leukemia are not considered to be toxicities (AE) and do not need to resolve to < Grade 1.

7) Prior DNA methyl transferase inhibitor administration (azacitidine, decitabine or guadecitabine) for AML or for antecedent MDS is allowed if this clinical trial is considered the best treatment option, but azacitidine, decitabine or guadecitabine must have been stopped at least 3 weeks prior to Day 1 of treatment on the study.

8) Participants are required to stop receiving myeloid growth factors at least 1 week (Neupogen) or 2 weeks (Neulasta) before starting treatment on the study.

9) Age ≥18 years.
Because no dosing or adverse event data are currently available on the use of decitabine or talazoparib in participants <18 years of age, children are excluded from this study, but will be eligible for future pediatric trials.

10) ECOG performance status ≤2 (Karnofsky ≥60%, see Appendix A).

11) Life expectancy of greater than 2 months.

12) Participants must have normal organ function as defined below:

- Total bilirubin within normal institutional limits unless thought due to hemolysis or to Gilbert's syndrome;
- AST(SGOT)/ALT(SGPT) ≤2.5 × institutional upper limit of normal;
- Creatinine within normal institutional limits;

OR

- Creatinine clearance ≥60 mL/min/1.73 m^2 for participants with creatinine levels above institutional normal.

13) Female participants of childbearing potential must have a negative pregnancy test, and female participants of childbearing potential and male participants must agree to use adequate contraception.

Decitabine has been assigned to pregnancy category D by the FDA. Pregnant women must not take decitabine and female participants must immediately stop taking decitabine and inform their doctor if they become pregnant during treatment. Decitabine is expected to result in adverse reproductive effects and can cause fetal harm when administered to a pregnant woman. In preclinical studies in mice and rats, decitabine was teratogenic, fetotoxic, and embryotoxic.

Studies in pregnant animals to evaluate the effect of talazoparib on pregnancy have not been performed.

Because decitabine is known to be teratogenic and the effects of talazoparib on the developing human fetus are unknown, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect that she is pregnant while she or her partner is participating in this study, she should inform the treating physician immediately. Men treated or enrolled on this protocol must also agree to use adequate contraception prior to the study, for the duration of study participation, and 4 months after completion of decitabine and talazoparib administration.

It is not known whether decitabine is excreted in human milk. Similarly, studies in lactating animals to evaluate the effect of talazoparib have not been performed, and thus, it is not known whether talazoparib is excreted in human milk. Therefore, breast-feeding should be stopped during decitabine and talazoparib treatment.

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

3.2 Exclusion Criteria

1) Patients with acute promyelocytic leukemia.

2) Patients with active central nervous system leukemia or requiring maintenance intrathecal chemotherapy.

3) Patients receiving concurrent chemotherapy, radiation therapy, or immunotherapy for AML.

4) Patients receiving any other investigational agents.

5) Hyperleukocytosis with >50,000 blasts/µl. Hydroxyurea for blast count control is permitted before starting treatment, but must be stopped at least 24 hours prior to starting treatment on the study. Patients will be withdrawn from the study if > 50,000 blasts/µl occur or recur > 14 days after starting treatment on the study.

6) Active, uncontrolled infection. Patients with infection controlled with antibiotics are eligible.

7) Uncontrolled intercurrent illness including, but not limited to, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that per investigator's judgment would limit compliance with study requirements.

8) Patients who are pregnant or nursing.

9) Patients who have had chemotherapy within 3 weeks (6 weeks for nitrosoureas or mitomycin C) or radiotherapy within 2 weeks for antecedent malignancies prior to entering the study, or those who have not recovered from adverse events due to agents administered more than 4 weeks earlier.

10) History of allergic reactions attributed to compounds of similar chemical or biologic

composition to decitabine or talazoparib.

11) Known HIV infection.

HIV-positive patients are at increased risk of lethal infections when treated with marrow-suppressive therapy. Appropriate studies will be undertaken in patients receiving combination antiretroviral therapy when indicated.

12) Previous treatment with talazoparib.

3.3 Inclusion of Women and Minorities

Men and women of all races and ethnic groups are eligible for this trial.

4. REGISTRATION PROCEDURES

Informed consent and HIPAA authorization will be obtained prior to screening of potentially eligible patients. Patients will sign a statement of informed consent that meets the requirements of the Code of Federal Regulations Title 21, International Conference on Harmonization (ICH) E6 Good Clinical Practices, and the local Institutional Review Board (IRB). Prior to pre-study procedures, a unique ID number will be assigned to the participant.

Once the signed informed consent has been obtained, pre-study evaluation studies will be performed. After the pre-study evaluation studies are completed, eligibility of the participant will be reviewed. Eligible participants will be enrolled on the study. Decitabine and talazoparib dose levels will be assigned.

Participants should begin protocol treatment within 14 days of signing consent.

To register a participant, the following information should be completed/compiled by the research coordinator/data manager and faxed to 410-328-1180 or e-mailed to the Coordinating Center Contact, Gary Saum, at gary.saum@umm.edu:

- Signed informed consent form;
- HIPAA authorization form (if not part of consent);
- Institution name;
- Treating physician;
- Copy of required laboratory tests (source documentation for eligibility and pre-study procedures) including pathology report (if pathology report not immediately available, the investigator must sign the eligibility worksheet confirming diagnosis and submit the pathology report when available);
- Eligibility screening worksheet; and
- Registration form.

The research coordinator or data manager at the participating site will then call 410-328-7680 or e-mail Gary Saum (gary.saum@umm.edu), the Coordinating Center Contact, to verify eligibility.

To complete the registration process, the Study Coordinator or Coordinating Center Contact at the Coordinating Center will:

- Assign a participant study ID number;
- Register the participant on the study;
- Assign decitabine and talazoparib dose levels;
- Fax or e-mail the participant study number and dose levels to the participating site; and
- Call the research coordinator or data manager at the participating site and verbally confirm registration.

A participant may not start treatment on the study until registration is confirmed by the Coordinating Center.

5. TREATMENT PLAN

5.1 Agent Administration

Treatment may be administered on an outpatient or inpatient basis.

Table 1. Regimen description

Regimen Description					
Agent	Premedications; Precautions	Dose	Route	Schedule	Cycle Length
Decitabine	Premedicate with antiemetic daily prior to decitabine	**	IV	Days 1-5	28 days (4 weeks ± 3 days)
Talazoparib	Can be administered regardless of food intake.	**	PO	Days 1-28	
**Doses as appropriate for assigned dose level.					

- Talazoparib orally Days 1-28 (± 3 days) at the same time each morning.
- Decitabine should be administered at a consistent time, after talazoparib.

NOTE: Participants will be requested to maintain a medication diary (Appendix C) of each dose of talazoparib. The medication diary will be returned to study staff at the end of each cycle.

Reported adverse events and potential risks are described in Section 7.

Dose modifications are described in Section 6.

No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's AML.

5.1.1 Decitabine

Decitabine is administered intravenously (IV) daily for 5 or 10 days every 28 days (± 3 days). An antiemetic should be taken prior to each decitabine dose and, if needed, following administration of decitabine.

5.1.2 Talazoparib

Talazoparib is taken orally (PO) daily Days 1-28 (± 3 days) at the same time each day, before decitabine administration. It can be taken regardless of food intake.

Participants will be requested to maintain a medication diary of each dose of talazoparib. The medication diary will be returned to study staff at the end of each 28-day cycle.

5.1.3 Other Modalities or Procedures

None

5.2 Phase 1 Dose Escalation

5.2.1 Dose Escalation Schedule

Table 2. Dose escalation schedule

		Talazoparib (mg)*				
		0.25	0.50	0.75	1.0	1.0
Decitabine* (mg/m^2)	10	1	1a	1b	1c	-
	15	2	2a	2b	2c	-
	20	3	4	5	6	7**

***NOTE: DECITABINE IS DOSED IN MG/M^2, BUT TALAZOPARIB IS DOSED IN MG.**

** Cohort 7 will receive 20 mg/m2 of decitabine for 10 days and 1 mg of Talazoparib continuously.

The 'outer layer' of this nested dose escalation trial will escalate the dose of the two drugs by sequentially going through dose levels 1-6 in the table. The standard algorithm of the 3+3 design will be applied as specified below.

If the MTD has not been reached at dose level 6, the RP2D of decitabine will be 20 mg/m^2 combined with a dose of talazoparib of 1.0 mg. If 2 or more participants develop DLT at dose level 1, the combination of decitabine and talazoparib will be considered too toxic for further clinical development.

If the *provisional* MTD for decitabine in combination with talazoparib is dose level 1 or 2, one or the other of the 'inner layers' of the trial will be activated. If the provisional MTD corresponds to level 2, the dose of decitabine will be fixed at 15 mg/m^2 and the dose of talazoparib will be escalated through steps 2a-c, again using the algorithm of the 3+3 design below. If the tolerance is not exceeded at level 2c, then the RP2D of decitabine is 15 mg/m^2 combined with a dose of talazoparib of 1.0 mg.

Similarly, if the provisional MTD for the combination of the two agents from the outer layer is level 1, the dose of decitabine will be fixed at 10 mg/m^2 and the dose of talazoparib will be escalated through steps 1a-c, again using the algorithm of the 3+3 design below to define the MTD of the combination.

Phase 1 was originally planned through Cohort 6 above. In Cohorts 1 through 5, the study has enrolled almost exclusively patients previously treated with decitabine and/or azacitidine. The combination regimen has been very well tolerated, but response rates have been low. Given

excellent tolerability and low response rate, and given favorable results of 10-day decitabine in several series (12-14), we have opted to expand the Phase 1 component of the study with a 10-day decitabine cohort, Cohort 7, and, following completion of dose escalation, to enroll an expansion cohort of DNMTi-naïve patients at the RP2D. The statistical design has been updated as follows:

If Cohort 6, with 20 mg/m^2 decitabine daily for 5 days and talazoparib 1 mg daily, is completed without dose-limiting toxicity, Cohort 7, consisting of decitabine, 20 mg/m^2 daily for 10 days and talazoparib, 1 mg daily, will be added.

Following completion of Cohort 6 or Cohort 7 without dose-limiting toxicity, a minimum of 9 and a maximum of 17 patients not previously treated with DNMTis (decitabine, azacitidine and/or guadecitabine) will be enrolled to assess response in this patient population at the RP2D.

The aim of the expansion cohort will be to expand the safety data and to look for signs of activity in the DNMTi-naïve population.

Let P be the response rate of the combination at the RP2D. If we define a 'poor' response rate as P0=5% and a 'good' response rate as P1=25%, then we can test the null hypothesis that P≤P0 versus H1: P≥P1 with α=0.05 and power (1-β) of 80% using Simon's two-stage design. If we enroll 9 patients and see no responses, we can stop early for futility. If we see at least one response, we would then enroll an additional 8 patients in the second stage for a total of 17 patients. If we see 3 or more responses among the 17 patients, we can reject that the response rate is 5% or less with an error rate of 0.043. If we see 2 or fewer responses, we can reject the hypothesis that P≥25% with an actual (type II) error rate of 19.7%.

5.2.2 Definition of Dose-Limiting Toxicity

Dose-limiting toxicities will be evaluated in Cycle 1.

Toxicity will be evaluated according to Version 4.0 of the NCI Common Terminology Criteria for Adverse Events (CTCAE). Dose-limiting toxicity will consist of any of the following adverse events with an attribution of possible, probable, or definitely related to the study therapy:

- Any grade 4 treatment-related non-hematologic toxicity (except for infection, fever, neutropenic fever or bleeding, which are expected in this patient population).
- Any grade 3 treatment-related non-hematologic toxicity (except for infection, fever, neutropenic fever or bleeding, which are expected in this patient population) that does not resolve to ≤ grade 2 within 48 hours.
- Myelosuppression will be considered a dose-limiting toxicity if ANC <0.5 x 10^9/L and platelets <20 x 10^9/L (untransfused) persist on day 43, with bone marrow cellularity of ≤ 5% and no evidence of leukemia in the bone marrow.

Management and dose modifications associated with the above adverse events are outlined in Section 6.

5.2.3 Dose Escalation

Dose escalation will proceed within each cohort according to the following scheme. Dose-limiting

toxicity (DLT) is defined above.

Table 3. Dose escalation decision rules

Number of Participants with DLT at a Given Dose Combination Level	Escalation Decision Rule
0 out of 3	Enter 3 participants at the next dose combination.
≥2	Dose escalation will be stopped. This dose combination will be declared the maximally administered dose (highest dose administered). Three (3) additional participants will be entered at the next lowest dose combination if only 3 participants were treated previously at that dose.
1 out of 3	Enter at least 3 more participants at this dose combination. • If 0 of these 3 participants experience DLT, proceed to the next dose combination. • If 1 or more of this group experience DLT, then dose escalation is stopped, and this dose combination is declared the maximally administered dose combination. Three (3) additional participants will be entered at the next lowest dose combination if only 3 participants were treated previously at that dose combination.
≤1 out of 6 at highest dose combination level below the maximally administered dose combination	This is generally the recommended phase 2 dose combination. At least 6 participants must be entered at the recommended phase 2 dose combination.

5.3 Phase 2

Decitabine is administered intravenously (IV) daily for 5 or 10 days every 28 days (± 3 days). An antiemetic should be taken prior to each decitabine dose.

Talazoparib is administered orally daily Days 1-28 (± 3 days) at the same time each morning, before decitabine administration.

Phase 2 doses will be determined based on data from Phase 1, as detailed in the statistical section.

5.4 General Concomitant Medication and Supportive Care Guidelines

5.4.1 Antiemetic Therapy

Antiemetic therapy should be administered prior to decitabine, and, if needed, following administration of decitabine. The following antiemetics are recommended prior to each dose of decitabine and on an as needed basis: ondansetron (4-16 mg PO/IV) or equivalent $5HT_3$ antagonist. However, at the discretion of the treating physician, other anti-emetics may be administered, such as prochlorperazine 5-10 mg PO/IV every 6-8 hours, promethazine 12.5-25 mg PO/IV every 6 hours, or lorazepam 0.5-1 mg every 6 hours.

5.4.2 Tumor Lysis Prophylaxis and Management

To prevent the occurrence and complications of tumor lysis-induced hyperuricemia, all

participants without known allergy may receive allopurinol (zyloprim) 300 mg orally once a day starting on or before Day 1 and continuing until tumor lysis has abated. If a participant cannot take allopurinol, febuxostat (Uloric) 40 mg orally once a day can be prescribed. Rasburicase can be administered at the discretion of the treating physician to acutely lower uric acid levels, in conjunction with initiation of allopurinol or febuxostat to block uric acid formation. G6PD levels should be checked in appropriate participants, such as Black men, before rasburicase administration.

For individuals at risk for tumor lysis-induced hyperphosphatemia, phosphate binders (such as PhosLo, Amphojel, Renagel) can be administered every 4-6 hours starting on or before Day 1 and should continue as needed until tumor lysis has abated.

5.4.3 Infection Prophylaxis

Participants may receive prophylaxis directed against bacterial, viral and/or fungal infections, according to institutional practices, at the discretion of the principal investigator at the treating institution and the treating physician.

5.4.4 Hematopoietic Growth Factors

Participants are required to stop receiving myeloid growth factors at least 1 week (Neupogen) or 2 weeks (Neulasta) before starting treatment on the study. During the cycle of treatment, use of myeloid growth factors is permitted according to American Society of Clinical Oncology (ASCO) guidelines for participants with prognostic factors predictive of clinical deterioration and at the discretion of the treating physician. The use of growth factors must be recorded as concomitant medications.

5.5 Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment should continue on an ongoing basis until one of the following criteria applies:

- Disease progression;
- Intercurrent illness that prevents further administration of treatment;
- Unacceptable adverse event(s);
- Participant decides to withdraw from the study; or
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the investigator.

There is no maximum number of treatment cycles.

5.6 Criteria for Removal from Study

Participants will be removed from study when any of the criteria listed in Section 5.5 applies. The reason for study removal and the date the participant was removed must be documented in the Case Report Form.

5.7 Duration of Follow-up

Participants will be followed for 4 weeks after removal from study, then they will be followed every 6 months until death. Participants removed from study for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

6. DOSING DELAYS/DOSE MODIFICATIONS

Timing of cycle initiation and dose levels of decitabine and talazoparib starting with Cycle 2 will be guided by neutrophil/platelet counts after the prior cycle and peripheral blood (PB) and/or bone marrow (BM) blast percentage, as shown in Table 4, which describes dose modification and delay based on Day 28 or later PB counts starting with Cycle 2.

The treating physician may delay study drug treatment (decitabine and talazoparib) on Day 29 of any cycle regardless of neutrophil/platelet counts and/or leukemic blasts if the treating physician deems it in the patient's best interest to delay treatment. Treatment should be resumed at such time that it is deemed in the patient's best interest to do so. Reasons for delaying and for restarting should be documented in the medical record.

Bone marrow aspirate and biopsy will be performed on day 25-29 of cycle 1, and on day 25-29 of each subsequent treatment cycle unless circulating blasts persist in the peripheral blood, until documentation of CR or CRi. Bone marrow aspirate and biopsy will also be performed as needed to evaluate lack of count recovery (neutrophils <1000/μL and/or platelets <50,000/μ) by day 43) in the absence of blood blasts

Missed Doses
IV administration of the first 5 doses of Decitabine must be given within the first 7 days of the 28-day cycle. IV administration of the 10 doses of Decitabine must be given within the first 12 days of the 28-day cycle.

Talazoparib is taken orally on Days 1-28 of each cycle at the same time each morning. Missed morning doses may be taken later in the day. If a full day is missed, do not double up the next day.

Table 4. Decitabine and talazoparib dosing adjustment guidelines based on end of cycle ≥1 (C≥1, Day 29 or later) peripheral blood blasts and counts and bone marrow leukemic blasts (C≥1, Day 43)**

	Day 29 (+/- 4 days)			Day ≥43	
	Leukemic blasts in blood	**No leukemic blasts in blood, but leukemic blasts in bone marrow**	**No leukemic blasts in blood or marrow**	**Leukemic blasts in blood or marrow on Day 43**	**No leukemic blasts in blood or marrow and counts <thresholds**
Neutrophils <1000/µL or Platelets <50,000/µL	Administer both decitabine and talazoparib full doses, on schedule.	Administer both decitabine and talazoparib full doses, on schedule.	Delay decitabine and talazoparib until counts >threshold*, then administer decitabine and talazoparib full doses if ≤Day 43.	Administer both decitabine and talazoparib full doses.	Hold decitabine and talazoparib until counts >threshold*, then decrease both decitabine and talazoparib by one dose level.
Neutrophils ≥1000/µL and Platelets ≥50,000/µL	Administer both decitabine and talazoparib full doses, on schedule.	Administer both decitabine and talazoparib full doses, on schedule.	Administer both decitabine and talazoparib full doses, on schedule.	Administer both decitabine and talazoparib full doses.	Administer both decitabine and talazoparib full doses.

Threshold = Neutrophils ≥1000/µL and Platelets ≥50,000/µL

**** The treating physician may delay study drug treatment (decitabine and talazoparib) on Day 29 of any cycle regardless of neutrophil/platelet counts and/or leukemic blasts if the treating physician deems it in the patient's best interest to delay treatment. Treatment should be resumed at such time that it is deemed in the patient's best interest to do so. Reasons for delaying and for restarting should be documented in the medical record.

Decitabine dose levels are shown in Table 5.

Talazoparib dose levels are shown in Table 6.

Table 5. Decitabine dose levels

Decitabine (mg/m2)
10*
15
20

**Minimum dose allowed*

Table 6. Talazoparib dose levels

Talazoparib (mg)
0.25*
0.5
0.75
1

**Minimum dose allowed*

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

7.1 Comprehensive Adverse Events and Potential Risks Lists (CAEPRs)

7.1.1 CAEPRs for Decitabine

In a single-arm MDS study (N=99) DECITABINE was dosed at 20 mg/m2 intravenous, infused over one hour daily for 5 consecutive days of a 4 week cycle. Table 7 presents all adverse events regardless of causality occurring in at least 5% of participants.

Table 7. Adverse Events Reported in ≥5% of Participants in a Single-arm Study*

	DECITABINE N=99 (%)
Blood and lymphatic system disorders	
Anemia	31 (31%)
Febrile neutropenia	20 (20%)
Leukopenia	6 (6%)
Neutropenia	38 (38%)
Pancytopenia	5 (5%)
Thrombocythemia	5 (5%)
Thrombocytopenia	27 (27%)
Cardiac disorders	
Cardiac failure congestive	5 (5%)
Tachycardia	8 (8%)
Ear and labyrinth disorders	
Ear pain	6 (6%)
Gastrointestinal disorders	
Abdominal pain	14 (14%)
Abdominal pain upper	6 (6%)
Constipation	30 (30%)
Diarrhea	28 (28%)
Dyspepsia	10 (10%)
Dysphagia	5 (5%)
Gastro-esophageal reflux disease	5 (5%)
Nausea	40 (40%)
Oral pain	5 (5%)
Stomatitis	11 (11%)
Toothache	6 (6%)
Vomiting	16 (16%)

General disorders and administration site conditions	
Asthenia	15 (15%)
Chest pain	6 (6%)
Chills	16 (16%)
Fatigue	46 (46%)
Mucosal inflammation	9 (9%)
Edema	5 (5%)
Edema peripheral	27 (27%)
Pain	5 (5%)
Pyrexia	36 (36%)
Infections and infestations	
Cellulitis	9 (9%)
Oral candidiasis	6 (6%)
Pneumonia	20 (20%)
Sinusitis	6 (6%)
Staphylococcal bacteremia	8 (8%)
Tooth abscess	5 (5%)
Upper respiratory tract infection	10 (10%)
Urinary tract infection	7 (7%)
Injury, poisoning and procedural complications	
Contusion	9 (9%)
Investigations	
Blood bilirubin increased	6 (6%)
Breath sounds abnormal	5 (5%)
Metabolism and nutrition disorders	
Anorexia	23 (23%)
Decreased appetite	8 (8%)
Dehydration	8 (8%)
Hyperglycemia	6 (6%)
Hypokalemia	12 (12%)
Hypomagnesemia	5 (5%)
Musculoskeletal and connective tissue disorders	
Arthralgia	17 (17%)
Back pain	18 (18%)
Bone pain	6 (6%)
Muscle spasms	7 (7%)
Muscular weakness	5 (5%)
Musculoskeletal pain	5 (5%)
Myalgia	9 (9%)
Pain in extremity	18 (18%)

Nervous system disorders	
Dizziness	21 (21%)
Headache	23 (23%)
Psychiatric disorders	
Anxiety	9 (9%)
Confusional state	8 (8%)
Depression	9 (9%)
Insomnia	14 (14%)
Respiratory, thoracic and mediastinal disorders	
Cough	27 (27%)
Dyspnea	29 (29%)
Epistaxis	13 (13%)
Pharyngolaryngeal pain	8 (8%)
Pleural effusion	5 (5%)
Sinus congestion	5 (5%)
Skin and subcutaneous tissue disorders	
Dry skin	8 (8%)
Ecchymosis	9 (9%)
Erythema	5 (5%)
Night sweats	5 (5%)
Petechiae	12 (12%)
Pruritus	9 (9%)
Rash	11 (11%)
Skin lesion	5 (5%)
Vascular disorders	
Hypertension	6 (6%)
Hypotension	11 (11%)

* In this single arm study, investigators reported adverse events based on clinical signs and symptoms rather than predefined laboratory abnormalities. Thus not all laboratory abnormalities were recorded as adverse events.

Discussion of Clinically Important Adverse Reactions

In the single-arm study (N=99) when DECITABINE was dosed at 20 mg/m2 intravenous, infused over one hour daily for 5 consecutive days, the highest incidence of Grade 3 or Grade 4 adverse events were neutropenia (37%), thrombocytopenia (24%) and anemia (22%). Seventy-eight percent of participants had dose delays, the median duration of this delay was 7 days and the largest percentage of delays was due to hematologic toxicities. Hematologic toxicities and infections were the most frequent causes of dose delays and discontinuation. Eight participants had fatal events due to infection and/or bleeding (seven of which occurred in the clinical setting of myelosuppression) that were considered at least possibly related to drug treatment. Nineteen of 99 participants permanently discontinued therapy for adverse events.

No overall difference in safety was detected between participants >65 years of age and younger participants in these myelodysplasia trials. No significant gender differences in safety or efficacy

were detected. Participants with renal or hepatic dysfunction were not studied. Insufficient numbers of non-white participants were available to draw conclusions in these clinical trials.

Serious Adverse Events that occurred in participants receiving DECITABINE regardless of causality, not previously reported in **Table 7** include:

- Blood and Lymphatic System Disorders: myelosuppression, splenomegaly.
- Cardiac Disorders: myocardial infarction, cardio-respiratory arrest, cardiomyopathy, atrial fibrillation, supraventricular tachycardia.
- Gastrointestinal Disorders: gingival pain, upper gastrointestinal hemorrhage.
- General Disorders and Administrative Site Conditions: chest pain, catheter site hemorrhage.
- Hepatobiliary Disorders: cholecystitis.
- Infections and Infestations: fungal infection, sepsis, bronchopulmonary aspergillosis, peridiverticular abscess, respiratory tract infection, pseudomonal lung infection, Mycobacterium avium complex infection.
- Injury, Poisoning and Procedural Complications: post procedural pain, post procedural hemorrhage.
- Nervous System Disorders: intracranial hemorrhage.
- Psychiatric Disorders: mental status changes.
- Renal and Urinary Disorders: renal failure, urethral hemorrhage.
- Respiratory, Thoracic and Mediastinal Disorders: hemoptysis, lung infiltration, pulmonary embolism, respiratory arrest, pulmonary mass.
- Allergic Reaction: Hypersensitivity (anaphylactic reaction) to DECITABINE has been reported in a Phase 2 trial.

The following adverse reactions have been identified during post-approval use of DECITABINE. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure.

Cases of Sweet's Syndrome (acute febrile neutrophilic dermatosis) have been reported.

7.1.2 CAEPRs for Talazoparib

Two clinical studies were completed and three studies were ongoing at the time of protocol authorship. In the completed food effect study (673-203), single-dose talazoparib (500 µg) was safe and well tolerated in healthy male volunteers.

In the completed Study PRP-002 in advanced hematological malignancies and ongoing Study PRP-001 in advanced tumors, dose-limiting toxicities were observed at 900 µg/day and at 900 and 1100 µg/day, respectively, at the time of protocol authorship. Grade 3-4 events of anemia, neutropenia, and thrombocytopenia occurred in both studies and are being monitored closely throughout the clinical program.

The Phase 1 study (enrollment closed September 2014), PRP-001, initiated 03 January 2011, is an open-label, single-arm study evaluating talazoparib in adults with advanced tumors. The most commonly reported AEs were anemia, fatigue, and nausea. There were eight deaths assessed unrelated to study treatment.

The Phase 2 study, 673-201, initiated 08 May 2014, is an open-label, two-stage, two-cohort study evaluating talazoparib in adult females with locally advanced or metastatic breast cancer. Anemia,

neutropenia, and fatigue have each been reported for two of the four participants enrolled as of 30 November 2014. No SAEs or withdrawals from treatment have occurred.

The Phase 3 study, 673-301, initiated 30 October 2013, is an open-label, randomized, parallel-group, two-arm study of talazoparib versus protocol-specific physician's choice therapy in participants with germline BRCA mutation locally advanced and/or metastatic breast cancer. Of the 54 participants enrolled as of 30 November 2014, it is presumed about 36 were receiving talazoparib and about 18 were receiving another single-agent chemotherapy, based upon the 2:1 randomization scheme, but which participants were receiving which treatment is unknown. A Grade 3 pleural effusion requiring hospitalization resolved after 5 days. Two participants died due to disease progression and one participant died of neutropenic sepsis related to treatment, an identified risk for talazoparib (and for most comparator chemotherapies). Anemia, fatigue, nausea, headache, and thrombocytopenia have been the most commonly reported AEs. Three participants experienced SAEs that resolved without sequelae and resulted in no withdrawals from study treatment: leukopenia, anemia, and neutropenia (one participant), decreased neutrophil count (one participant), and atrial flutter (one participant).

7.2 Adverse Event Characteristics

Adverse Events
According to the ICH definition, an adverse event (AE) is any untoward medical occurrence in a participant or clinical investigation subject administered a pharmaceutical product, and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the investigational product.

This definition includes intercurrent illnesses or injuries that represent an exacerbation (increase in frequency, severity, or specificity) of pre-existing conditions. Whenever possible, it is preferable to record a diagnosis as the AE term rather than a series of terms relating to a diagnosis.

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site *http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm*.

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.

Serious Adverse Event (SAE) is defined as any of the following:

- Any death that occurs while the participant is enrolled in the study including the follow-up period or within 30 days of completing the study
- Immediately life-threatening adverse event
- Requires inpatient hospitalization

- Prolongation of an existing hospitalization
- Congenital anomaly/birth defect
- Medically important event
- Disability/incapacity (persistent or significant)

7.3 Reporting of Adverse Events Experiences

Adverse event information will be collected in an ongoing fashion through participant reporting AEs to their physician or health care provider. Seriousness and relatedness will be assessed by the treating physician, with appropriate reporting. Investigators are responsible for monitoring the safety of participants who have entered this study and for alerting the UMGCCC Data and Safety Monitoring and Quality Assurance Committee (DSM/QAC) to any event that seems unusual, even if this event may be considered an unanticipated benefit to the participant. Adverse events should be recorded in source documents and captured on case report forms.

A designated primary contact person based at the treatment center will be responsible for the collection and reporting of AEs for participants participating in the program.

7.3.1 Expedited/Serious Adverse Event Reporting

A serious adverse event (SAE) is defined as any AE that:

- Results in death
- Is life threatening, that is, places the participant at immediate risk of death from the event as it occurred. This definition does not include a reaction that, had it occurred in a more severe form, might have caused death
- Requires in-patient hospitalization or prolongation of an existing in-patient hospitalization. Admission of a participant to the hospital as an in-patient as a result of an AE, even if the participant is released on the same day, qualifies as hospitalization
- Results in persistent or significant disability or incapacity. An event qualifies as resulting in a persistent or significant disability or incapacity if it involves a substantial disruption of the participant's ability to carry out usual life functions. This definition is not intended to include experiences of relatively minor or temporary medical significance.
- Is a congenital anomaly or birth defect, an AE that occurs in the child or fetus of a participant exposed to the product prior to conception or during pregnancy
- Important medical event that does not meet any of the above criteria, but may jeopardize the participant or require medical or surgical intervention to prevent one of the outcomes listed above.

More than one of the above criteria may apply to any specific event.

All events that meet the definition of a serious adverse experience (SAE) or a drug safety and surveillance event (i.e pregnancy) which occur at any time during the clinical study or within 30 days of receiving the last dose of study drug(s), whether or not related to the study drug(s), must be reported by the Principal Investigator or designee to the Sponsor. FDA Form 3500A (MedWatch form) should be used to report the SAE to the Sponsor within 24 hours of site event notification using both of the Sponsor contacts below:

Attn: Maria Baer, MD	**Attn: Gary Saum, BA**
Fax: 410-328-6896	**Fax: 410-328-6896**
Email: mbaer@umm.edu	**Email: gary.saum@umm.edu**

The MedWatch form should also be used to report new or corrected event information. In addition, sites should report the event to their local Institutional Review Board per institutional policy.

Serious, related, unexpected adverse events will be reported to the FDA by the Sponsor within 7 days of unexpected and fatal/life threatening events, and within 15 days for unexpected and serious events as required by 21CFR312.32. If the serious adverse event or event of clinical interest (i.e. pregnancy) meets the threshold for FDA or drug safety and surveillance reporting, the Sponsor will forward the event information to FDA and Pfizer, Inc.

Pregnancy
Pregnancy in a participant being treated with the product should be reported immediately (within 24 hours of becoming aware of the pregnancy) to the sponsor by using the FDA 3500A (MedWatch Form). Every effort should be made to follow the participant through resolution of the pregnancy (termination or delivery) and report the resolution of the FDA 3500A (MedWatch Form) to the sponsor. The sponsor will forward the event information to FDA and Pfizer, Inc.

The Investigator/Sponsor will provide Pfizer, Inc., with a copy of this report.

Note: A death on study requires both routine and expedited reporting regardless of causality, unless as noted below. Attribution to treatment or other cause must be provided.

The Site Principal Investigator is responsible for appropriate medical care of study participants during the study in connection with protocol procedures. After a study participant's completion of or discontinuation from the study, the investigator remains responsible to follow, through an appropriate health care option, adverse events that are serious or that caused the study participant to discontinue before completing the study.

Reporting to Pfizer, Inc.

Serious Adverse Event Reporting:

Within 24 hours of awareness of a serious adverse event, whether or not related to the study drug, the Site Investigator will complete and submit a Medwatch 3500A Form to the Sponsor containing all required information (reference 21 CFR 312.32). The Sponsor will then submit a copy of this MedWatch 3500A form to the Pfizer, Inc. Safety Evaluation & Reporting fax, within the same timeframe.

The contact information for Sponsor submission of SAEs and 24-hour reports for events of overdose and specified liver test abnormalities is as follows:

Name: Safety Evaluation & Reporting.
Fax: 1-866-997-8322
Phone: 1-800-752-9737

The initial report should include, at minimum, the following:
- Study number (673-301)
- Site name and number
- Investigator name
- Participant number, sex, and age
- Details of study drug administration
- The date of the report

· A description of the SAE (event term, seriousness of the event)
· Causal relationship to the study drug

Follow-up information for the event should be sent to the Sponsor promptly (within 7 days) as necessary.

All serious adverse events (SAEs) and pregnancy reports whether or not considered drug-related should be reported to Pfizer, Inc.(via Safety Evaluation and Reporting contact information below) within 24 hours of receipt by the Sponsor, using the FDA 3500A (MedWatch Form) provided by the site.

The period during which all non-serious AEs and SAEs will be reported begins after informed consent is obtained and will continue through 30 days after of the last study visit or 30 days after the last dose of study medication, whichever comes first.

All non-serious adverse events whether or not considered drug-related should be reported to Pfizer, Inc.on a monthly basis by the Sponsor via a line listing which includes the following information: *Participant ID number, Date of Birth/Age, Gender, adverse event, onset date, outcome, resolution date (if applicable), severity, seriousness, action taken with study drug and causality*. Therefore, all Site Investigators are responsible for submitting all non-serious adverse events whether or not considered drug-related via a line listing, to the sponsor, inclusive of the same information listed above.

Site clinicians should not wait to collect additional information that fully documents the event before notifying the Sponsor of an SAE or pregnancy. The Sponsor may be required to report certain SAEs to regulatory authorities within 7 calendar days of being notified about the event; therefore it is important that site clinicians submit additional information to the sponsor as soon as it becomes available.

Product Complaints Reporting

A product complaint (“Complaint”) is any direct, written, electronic, or oral communication of dissatisfaction that alleges deficiencies related to the identity, quality, durability, labeling, purity, stability, appearance, effectiveness, safety, and/or design of a drug product after it is released for distribution.

Complaints that simultaneously fall under Adverse Event definitions under this Protocol need only be reported via the Adverse Event reporting procedure set forth in this Protocol.

Investigator or designee (Reporter) shall capture the following Complaint information as relates to talazoparib used under this Protocol:

- Date complaint received
- Product Name and Lot Number
- Indicate if the product is available for return to Pfizer, Inc. for investigation
- Quantity Affected
- Detailed Description of complaint
- Study Protocol Number
- Investigator Name

- Site Contact
- Site Number
- Participant Number
- Name and contact information of the person who is reporting the complaint as well as name and contact information of the complainant. The reporter may be contacted by Pfizer, Inc..

The Investigator or designee (Reporter) will use his or her best efforts to report Complaints to Pfizer, Inc. within five days of learning of the Complaint.

Investigator or designee will submit the complaint information to Pfizer, Inc., as follows:

Name: Safety Evaluation & Reporting.
Fax: 1-866-997-8322
Phone: 1-800-752-9737

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational agents administered in this study can be found in *Sections 7.1 and 7.2*.

8.1 Decitabine

8.1.1 Drug Substance (Active Pharmaceutical Ingredient)

DECITABINE (decitabine) for Injection contains decitabine (5-aza-2'-deoxycitidine), an analogue of the natural nucleoside 2'-deoxycytidine.

Chemical Name (IUPAC):	4-amino-1-(2-deoxy-β-D-erythro-pentofuranosyl)-1,3,5-triazin-2(1*H*)-one
Chemical Formula:	C8H12N4O4
Molecular Weight:	228.21 Da

Decitabine is a fine, white to almost white powder. Decitabine is slightly soluble in ethanol/water (50/50), methanol/water (50/50) and methanol; sparingly soluble in water and soluble in dimethylsulfoxide (DMSO).

8.1.2 Drug Product and Formulation

DECITABINE (decitabine) for Injection is a white to almost white sterile lyophilized powder supplied in a clear colorless glass vial. Each 20 mL, single dose, glass vial contains 50 mg decitabine, 68 mg monobasic potassium phosphate (potassium dihydrogen phosphate) and 11.6 mg sodium hydroxide.

8.1.3 Packaging, Storage, and Handling

Decitabine, 50 mg single-dose vial individually packaged in a carton.

Store vials at 25°C (77°F); excursions permitted to 15-30°C (59-86°F).

Occupational Safety and Health Administration (OSHA) Guidelines for handling cytotoxic drugs outlined in the American Journal of Hospital Pharmacy should be followed (36). As with other potentially toxic anti-cancer agents, care should be exercised in the handling and preparation of decitabine. The use of gloves and protective garments is recommended. Preparation should occur in a vertical laminar flow biological hood using proper aseptic technique. For skin contact or spillage, refer to the material safety data sheet (MSDS) for treatment options.

8.1.4 Formulation, Dosage, and Administration

DECITABINE is administered at a dose of 20 mg/m2 by continuous intravenous infusion over 1 hour repeated daily for 5 or 10 days. This cycle should be repeated every 4 weeks. Participants may be premedicated with standard anti-emetic therapy.
If myelosuppression is present, subsequent treatment cycles of DECITABINE should be delayed until there is hematologic recovery (ANC ≥1,000/μL platelets ≥50,000/μL).

It is recommended that participants be treated for a minimum of 4 cycles; however, a complete or partial response may take longer than 4 cycles.

DECITABINE is a cytotoxic drug and caution should be exercised when handling and preparing DECITABINE. Procedures for proper handling and disposal of antineoplastic drugs should be applied. Several guidances on this subject have been published (36).

DECITABINE should be aseptically reconstituted with 10 mL of Sterile Water for Injection (USP); upon reconstitution, each mL contains approximately 5.0 mg of decitabine at pH 6.7-7.3. Immediately after reconstitution, the solution should be further diluted with 0.9% Sodium Chloride Injection or 5% Dextrose Injection to a final drug concentration of 0.1-1.0 mg/mL. Unless used within 15 minutes of reconstitution, the diluted solution must be prepared using cold (2˚C - 8˚C) infusion fluids and stored at 2˚C - 8˚C (36˚F - 46˚F) for up to a maximum of 4 hours until administration.
Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Do not use if there is evidence of particulate matter or discoloration.

DECITABINE (decitabine) for Injection is supplied as a sterile, lyophilized white to almost white powder, in a single-dose vial, packaged in cartons of 1 vial. Each vial contains 50 mg of decitabine.

8.1.5 Drug Supply

Decitabine will be purchased commercially and billed to insurance.

8.2 Talazoparib

8.2.1 Names

Code Name: BMN 673ts

Generic Name: Talazoparib tosylate
USAN Name: Talazoparib tosylate
INN Name: Talazoparib
Trade Name: None

8.2.2 Active Ingredient

The active ingredient is talazoparib tosylate, the tosylate salt of the active moiety, Talazoparib; the chemical name (8**S** ,9**R**) 5-fluoro-8-(4-fluorophenyl-2,7,8,9-tetrahydro-9-(1- methyl-1***H***-1,2,4-triazol-5-yl)- 3***H***-pyrido[4,3,2 de]phthalazin-3-one, 4 methylbenzenesulfonate (1:1). (Chemical Abstracts Service Nomenclature).

8.2.3 Pharmacological Class

Talazoparib is a PARP inhibitor.

8.2.4 Structural Formula

Talazoparib tosylate (salt):
Molecular Formula: C26H22F2N6O4S (C19H14F2N6O • C7H8O3S)
Molecular Weight: 552.5624
Talazoparib free base:
Molecular Formula: C26H22F2N6O4S
Molecular Weight: 552.5624
1.453 mg talazoparib tosylate is equivalent to 1.00 mg talazoparib free base.

8.2.5 Route of Administration

Oral administration.

8.2.6 Method of Manufacture

Talazoparib tosylate is manufactured by chemical synthesis in accordance with current Good Manufacturing Practices.

8.2.7 Formulation of the Study Drug

The drug product is a capsule formulation comprised of a blend of talazoparib tosylate drug substance and silicified microcrystalline cellulose filled into a hypromellose capsule. The capsules are presented in three strengths (0.10 mg, 0.25 mg, and 1.0 mg free base equivalent) distinguished by capsule color or size and described in milligrams (mg) per capsule. Capsules used for initial clinical studies were presented in four strengths (25 µg, 50 µg, 250 µg, and 1.0 mg) and described in micrograms (µg) per capsule.

8.2.8 Storage, Labeling, and Destruction

The drug product is stored at room temperature (15º-30º C; 59º-86º F). The sponsor should be notified of any temperature excursions immediately. Capsules used for initial clinical studies were packaged in perforated unit-dose blister cards composed of cold- form aluminum foil and push-through aluminum lidding. Capsules used for subsequent clinical studies (0.10 mg, opaque ivory, size 4; 0.25 mg, opaque white, size 4; 1.0 mg, opaque flesh, size 4) are supplied in high-density

polyethylene bottles, with induction-sealed closures, containing 30 drug product capsules. The label on the packaging has instructions in English (or other language, as required) and may contain any or all of the following information: sponsor's name, study number, name of the investigational product, contents, recommended storage condition, a precautionary statement, product retest or expiration date, lot number, and participant ID.

Any returned, expired, or unused drug should be destroyed at the site, using the site's drug destruction SOP. Evidence of destruction must be sent to the Sponsor. Sites are to send SOP for drug destruction to the sponsor.

8.2.9 Agent Ordering

All sites will request their own site's supply of Talazoparib directly from Pfizer and Pfizer will arrange the supply via the Pfizer supply chain.

9. BIOMARKER AND CORRELATIVE STUDIES

9.1 Sample Collection

See Protocol Appendix B and the site laboratory manual for sample collection guidelines.

9.1.1 Peripheral blood

Up to 20 ml of peripheral blood for pharmacodynamics will be collected from participants on:

- Cycle 1, Day 1, before any treatment;
- Cycle 1, Day 5, one hour after administration of decitabine is completed, and
- Cycle 1, Day 8, anytime
- For participants receiving 10-day decitabine, sampling will be on Days 1, 5, 8 and 10, one hour after the decitabine injection.

Up to 10 mL of peripheral blood for epigenetic studies will be collected from participants on:

- Cycle 1, Day 1, before any treatment;
- Cycle 1, Day 5, one hour after administration of decitabine;
- Cycle 1, Day 8, anytime;
- For participants receiving 10-day decitabine, sampling will be on Days 1, 5, 8 and 10, one hour after the decitabine injection.
- Cycle 1, Day 29, (± 3 days) before any treatment; and
- Day 29 (± 3 days) of every cycle, before any treatment.

9.1.2 Bone Marrow (Epigenetic)

Up to 10mL of bone marrow collected on:

- Screening;
- Cycle 1, Day 29 (± 3 days) , before any treatment.
- Days 25-29 (± 3 days) of every cycle, before any treatment (unless circulating blasts persist in the peripheral blood), until documentation of CR or CRi.

9.1.3 Buccal Swab

Two buccal swabs will be collected at screening

9.1.4 Coding of Samples

The clinical study team will record the participant's unique study ID and the sample number in the in the participant research record.

Samples leaving the clinical sites will not be labeled with PHI.

9.2 Sample Processing

Samples will be processed as described below and according to the lab manual for specific assays. Sample collection prioritization is noted in Protocol Appendix D.

Unused portions of the samples will be aliquoted and stored according to VARI Biorepository standards for future, unplanned research.

9.3 Shipping of Samples

Samples will be sent from the clinical sites to the Rassool laboratory and to VARI. Please see the lab manual for shipping instructions. Secondary shipment may be necessary at the discretion of the PI for either unused portions of samples from the Rassool laboratory to VARI for banking, or from VARI to collaborating institutions for completion of the prescribed correlative assays below.

All shipping of specimens will be in compliance with Federal, state and local regulations and IATA standards.

9.4 Storage of Samples/Biobanking

Bone marrow, peripheral blood, and buccal swab samples collected for correlative studies performed in this protocol, and components from the processed cells, may be stored indefinitely to answer research scientific questions related to AML, study drugs, epigenetics, immune response and/or cancer. The Participant retains the right to have the sample material destroyed at any time by contacting the Principal Investigator and making the request in writing. If a participant withdraws from the study, their research samples will be kept and used as described elsewhere in the protocol unless the participant specifically requests their destruction in writing.

Samples will be stored at the clinical sites for no more than 2 weeks until shipped to the Rassool Laboratory or to VARI. The Rassool laboratory will send unused portions of the samples to VARI at completion of the study. The VARI Biorepository will serve as long-term, biobanking storage for the samples and unused sample derivatives.

9.5 Colony Formation, Immunofluorescence, PARP Trapping, mRNA and Protein Expression, and DNA DSB Repair.

The following processes will be conducted in the event that there is sufficient blood sample left over from the study. See Appendix D for sample analysis priorities.

As detailed in *section 2.4.4*, our mechanistic hypothesis is that decitabine and talazoparib will cooperate to increase the cytotoxic DNA-PARP complexes on chromatin, resulting in decreased DNA DSB repair. The following assays will test components of this mechanism:

1) Colony forming assays in methyl cellulose.
2) Immunostaining for γH2AX (a marker for DNA DSBs), DSB repair, including RAD51 (a major component of the homologous recombination pathway), epigenetic and DNA replication markers.
3) PARP trapping.
4) PAR activity
5) Protein and mRNA expression of DSB repair genes, including PARP1, PAR, RAD51, KU70/80, DNA ligase 3, and epigenetic factors, including EZH2 and DNMT1, by Western blot analysis
6) Levels of mRNA expression of DSB repair genes, including PARP1, RAD51, KU70/80 and DNA ligase 3, by quantitative polymerase chain reaction (Q-PCR)
7) Levels of DSB repair, measured using *in vitro* plasmid-based assays.

9.5.1 Collection of Samples

Please see lab manual for sample collection procedures.

9.5.2 Processing of Samples

Please see lab manual for sample processing procedures.

Peripheral blood mononuclear cells (MNC) will be isolated from AML samples by density centrifugation using Ficoll Hypaque (Sigma), according to the manufacturer's instructions. Differential cell counts will be performed after Wright-Giemsa staining, and recorded. MNCs will be sent to the Rassool laboratory as flash frozen pellets and as viably frozen cells. For flash frozen cells, MNCs will be centrifuged at 1500 rpm for x 5 mins and cell pellets will be flash frozen in liquid nitrogen for RT-PCR, western blotting, and PARP trapping analyses. For viably frozen cells, MNCs will be viably frozen in IMDM (Invitrogen) with 10% FBS and 5% DMSO for colony formation and immunofluorescence assays.

Details of assays are below. Note that these assays are subject to change based on experience with samples.

9.5.3 Colony-Forming Assays

a) Hypothesis and Rationale:

Colony numbers decrease in AML cells treated with talazoparib and decitabine.

b) Mechanistic Rationale:

Our recently published data show that AML cells treated with decitabine and/or the PARP inhibitor Talazoparib demonstrate decreased colony forming ability in methylcellulose (34). The mechanistic rationale is that the combination of decitabine and talazoparib will lead to increased cytotoxicity or anti-proliferative effects, resulting in decreased colony formation.

c) Intended Use Within this Study:

This study will determine whether colony formation is decreased in treated samples from participants, relative to pre-treatment samples.

d) Detailed Protocol:

Viably frozen MNCs will be, thawed, counted, and suspended in 0.4 mL HPGM (Lonza) so that a density of 100 thousand cells per 1.1 mL is obtained when the 0.4 mL cells suspension is mixed with 4 mL MethoCult (StemCell Technologies). Each sample will be plated in technical triplicates (100 thousand cells per well in 1.1 mL Methocult) on a non-tissue culture treated 6-well plate (Greiner Bio) and incubated for 10-14 days at 37^0C + 5% CO_2. At the end of the incubation period, colonies will be stained with 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenlytetrazolium chloride (1 mg/mL) overnight at 37^0C and counted using a colony counter (Synbiosis, Frederick, MD).

e) Quantification of Colony Numbers and Statistical Analysis:

Number of colonies from triplicates will be determined in samples taken during treatment relative to pre-treatment samples. Colony numbers for treatment samples will divided by colony numbers from respective pre-treatment samples, and expressed as a percentage of the pretreatment value. *Result reporting:* Results will be represented as average percentage colony formation relative to pre-treatment ± SEM. All statistical analyses will be performed using the Student t-test, with p-values ≤ 0.05 considered significant. We expect that colony numbers will be significantly decreased in Day 5 post-treatment AML samples, compared with Day 1 pre-treatment samples.

9.5.4 γH2Ax foci and DSB repair, including RAD51 (a major component of the homologous recombination pathway), **and co-localization of EZH2, DNMT1, DNA replication factors and other proteins** (Immunofluorescence).

a) Hypothesis and Rationale

Levels of γH2Ax foci will increase and RAD51 foci will decrease in AML cells treated with decitabine and talazoparib. Co-localization of epigenetic and/or DNA replication factors is expected with γH2Ax.

b) Mechanistic Rationale: Published reports demonstrate that γH2Ax is a marker for DNA damage and in particular, lethal DSBs (34) Unpublished data from our laboratory demonstrate that AML cells treated with decitabine at low, non-cytotoxic, concentrations show no change in levels of γH2AX, compared with untreated controls. In contrast, AML cells treated with talazoparib at low nanomolar concentrations or ABT888 at 500 nM demonstrate increased levels of γH2AX, compared with controls, and AML cells treated with combinations of decitabine and talazoparib or ABT888 have increased levels of γH2AX, compared with talazoparib or ABT888 alone. Furthermore, our preliminary data in AML cell lines suggest that homologous recombination (HR) is downregulated by DNMTis. RAD51 is a marker for homologous recombination that should

decrease with treatment. *Thus, the mechanistic rationale for the combination of* decitabine *and talazoparib is that the decitabine is reprogramming the epitranscriptome to down-regulate HR, including RAD51, leading to increased AML cell death.*

c) Intended Use Within this Study: This study will determine whether levels of cytotoxic DSBs increase in treated samples from AML participants (relative to pretreatment samples), as measured by formation of γH2AX foci and decrease of RAD51 foci (i.e. decreased HR), and whether these changes will correlate with clinical responses.

c) Intended Use Within this Study: This study will determine whether levels of cytotoxic DSBs increase in treated samples from AML participants (relative to pretreatment samples), as measured by γH2AX foci, whether RAD51 foci decrease (i.e. decreased HR), whether other epigenetic and DSB repair proteins co-localize with these foci, and whether these changes will correlate with clinical responses.

d) Assay Methods

i. Detailed Protocol: Viably frozen AML MNCs will be thawed and cytospun onto glass slides (0.2 X 10^6 cells/slide) for 5 minutes at 200 rpm in 1X PBS on a Shandon Cytospin 4. Cells will then be fixed for 30 minutes in 4% paraformaldehyde, washed 3 times in DPBS (+Cacl2 and MgCl2, DPBS++), permeabilized for 10 minutes in permeabilization solution (50mM NaCl, 3mM MgCl2, 10mM HEPES, 200mM Sucrose and 0.5% Triton X-100 in PBS 1X), washed 3 times in DPBS (+Cacl2, +MgCl2) supplemented with BSA 1% (DPBS-BSA) and then blocked overnight in DPBS++ supplemented with 10% serum. After incubation with mouse monoclonal anti-γH2A.x (1:100, Upstate) and rabbit polyclonal anti-RAD51 (1:100, Santa Cruz Biotechnologies, Dallas, TX) or isotype controls for one hour at 37^0C in DPBS-BSA, cells will be washed and then incubated with Dylight 594-anti-mouse and Dylight 488-anti rabbit (1:200, KPL, Gaithersburg, MD) for one hour at 37^0C prior to counterstaining with 4',6 diamidino-2-phenylindole, dihydrochloride (DAPI, 0.3 µg/ml, Promega, Madison, WI) in mounting medium (Vectashield, Vector Laboratories, Burlingame, CA). Slides will be examined using a Nikon fluorescent microscope Eclipse 80i (100X/1.4 oil, Melville, NY).

ii. Foci Quantitation: Images of cells with from at least 50 cells/slide will be captured using a CCD (charge-coupled device) camera and the imaging software NIS Elements (BR 3.00, Nikon). γH2AX and RAD51 foci contained within these imaged cells will be counted and the data will be expressed as numbers of foci per cell.

iii. Statistical Analysis and Result Reporting: Immunostaining for γH2AX and other proteins including RAD51 foci will be performed on MNCs from AML PB samples obtained on Day 1 pre-treatment and on Day 5 following treatment. γH2AX and other proteins including RAD51 foci will be quantitated as described in the foci quantitation section below and results will be compared with those of pretreated controls. We expect that γH2AX foci/cell will increase and other proteins including RAD51 foci will decrease in AML samples taken post-treatment on Day 5, compared with pre-treatment on Day 1. Results will be reported as the average number of foci/cell in each sample within an individual participant. The Student's t-test will be used to determine significant difference between pre-treatment and post-treatment samples, $p \leq 0.05$ considered significant.

iv. Correlation with Clinical Responses: γH2AX and other proteins including RAD51 and epigenetic factors in AML participant samples will be correlated with clinical responses, in

collaboration with Dr Søren Bentzen.

9.5.5 PARP Trapping

a) Hypothesis and Rationale

Levels of PARP trapping will increase in AML participant samples following treatment with talazoparib and decitabine, and increases in PARP trapping will correlate with clinical responses.

b) Mechanistic Rationale: PARP inhibitors trap PARP1 in chromatin as one of their mechanisms of action, and PARP trapping correlates with the cytotoxic effects of PARP inhibitors (16,29). The laboratory of Dr. Rassool has demonstrated that decitabine treatment also leads to trapping of PARP in chromatin in AML cells, and that combinations of decitabine and PARPis enhance PARP trapping, in relation to the effect of either drug alone (34). *Thus the mechanistic rationale for the combination of* decitabine *and talazoparib is that it will increase PARP trapping in chromatin, leading to increased AML cell death.*

c) Intended Use Within this Study: This study will determine whether PARP trapping is increased in chromatin fractions of AML blasts after 5 or 10 days of combined decitabine and talazoparib treatment, compared with pre-treatment samples. These results will also be correlated with participant responses.

d) Assay Methods

i. Detailed Protocol:

Method 1: Chromatin quantification: Flash frozen [2 vials] MNCs will be used to isolate chromatin fractions using the Subcellular Protein Fractionation kit (Thermo Fisher Scientific, Waltham, MA), following manufacturer instructions. Immunoblotting will then be performed following standard procedures using 5 ug of chromatin lysate. Proteins will be loaded onto 4-20% SDS-PAGE gel (Bio-Rad laboratories, Hercules, CA), transferred on PVDF membrane (GE Healthcare Life Sciences, Pittsburgh, PA) and blots will be washed in Tris Buffered Saline-0.1% Tween 20 (TBST) three times, blocked in TBST-5% bovine serum albumin (TBST-BSA) for an hour at least and first antibodies in TBST-BSA are applied overnight at 4ºC on a shaker. Blots will be washed again 3 times and secondary antibodies, anti-Mouse-horse radish peroxidase (HRP; Cell signaling, Danvers, MA) or anti-Rabbit-HRP (BioLegend, San Diego, CA) or alkaline phosphate (AP; Bio-Rad) in TBST are applied for one hour followed by 3 washes and detection of HRP using enhanced chemiluminescence or alkaline phosphatase using 5-bromo-4-chloro-3-indolyl-phosphate/nitro blue tetrazolium (BCIP/NBT; Promega). Blots will be probed for PARP1, using PARP1 mouse monoclonal (1:3000, C2-10, BD Biosciences) and DNMT1 using mouse monoclonal (1:5000, Imgenex, San Diego, CA). Histone H3 rabbit polyclonal (1:30000, Sigma-Aldrich) will be used as the loading control.

Method 2: Proximity ligation assay (PLA): The procedure will be performed using the Duolink In Situ assay kit (Sigma) according to manufacturer's instructions (sigma.com/duolink). Briefly, viably frozen AML MNCs will be processed as described in section 9.5.4 for immunofluorescence. Primary antibodies mouse anti-PARP1 (Sigma) and rabbit anti-H2Ax (Cell Signaling Technologies) will be used at 1:400 and incubated overnight at 4ºC, followed by 3 washes in PBS. Secondary antibodies conjugated to PLA plus and minus probes will be diluted 1:5 in antibody dilution buffer (included in kit) and slides incubated for 1hr at 37ºC followed by three washes in PBS. Ligation, amplification, and counterstaining are performed according to kit instructions. Slides will be examined using a Nikon fluorescent microscope

Eclipse 80i (100X/1.4 oil, Melville, NY).

ii. **Densitometry, Image analysis, Statistical Analysis and Result Reporting:** Protein levels on immunoblots will be quantitated by densitometry using Quantity-one software (v.4.6, Bio-Rad) after scanning nonsaturated luminograms. PLA fluorescent spots from at least 50 cells/slide will be captured using a CCD (charge-coupled device) camera and the imaging software NIS Elements (BR 3.00, Nikon). *Result reporting:* C1D1 Samples (pre-treatment) are set at 1 for PARP1 band density (determined using ImageJ Software [NIH]), and PARP trapping in treatment samples (C1D5 and C1D8) will be assessed relative to pre-treatment samples as fold change. For PLA, results will be reported as the average number of fluorescent spots per cell in each sample from an individual participant. Treatment samples will be compared to pre-treatment samples in three technical replicates (each sample will be analyzed three times) using the average of the logarithm of the fold change ± SEM. All statistical analyses will be performed using the Student's t-test on the log-transformed values, with p-values ≤ 0.05 considered significant.

iii. **Correlation with Clinical Responses:** PARP trapping results in AML participant samples will be correlated with clinical responses, in collaboration with Dr Søren Bentzen, Director of Biostatistics at the University of Maryland and of the Biostatistics Shared Service, UMGCCC.

9.5.6 DNA repair and epigenetic factors - Protein and mRNA Expression

a) Hypothesis and Rationale

Protein and mRNA levels of DNA repair and epigenetic factors decrease in soluble fractions from AML cells treated with talazoparib and decitabine.

b) Mechanistic Rationale: Cells treated with decitabine or azacitidine have decreased levels of DNMT1, 2 and 3a in soluble (non-chromatin) fractions (37). Our unpublished data show that soluble cellular lysates from AML cells treated with decitabine and/or the PARP inhibitor ABT888 demonstrate decreased levels of both DNA repair and epigenetic factors.. In addition, increased caspase cleavage is observed with the drug combination. In contrast, chromatin fractions demonstrate increased levels of PARP1.

The mechanistic rationale is that the combination of decitabine and the PARP inhibitor talazoparib will lead to decreased levels of these above factors in soluble (non-chromatin) cellular fractions, and, in contrast, increased levels of PARP1 in chromatin.

c) Intended Use Within this Study: This study will determine whether levels of these factors decrease in soluble cellular fractions, in contrast to increased PARP trapping in chromatin, in treated samples from AML participants, relative to pre-treatment samples.

d) Detailed Protocol

i. **Immunoblotting:** AML MNCs (flash frozen) will be used to isolate whole cell lysates, following standard procedures described previously (38,39). Immunoblotting will then be performed following standard procedures using 5 ug of whole cell lysate. Proteins will be loaded onto 4-20% SDS-PAGE gel (Bio-Rad laboratories, Hercules, CA), transferred onto

PVDF membrane (GE Healthcare Life Sciences, Pittsburgh, PA) and blots will be washed in Tris Buffered Saline-0.1% Tween 20 (TBST) three times, blocked in TBST-5% bovine serum albumin (TBST-BSA) for an hour at least and first antibodies in TBST-BSA are applied overnight at 4°C on a shaker. Blots will be washed again 3 times and secondary antibodies, anti-Mouse-horse radish peroxidase (HRP; Cell signaling, Danvers, MA) or anti-Rabbit-HRP (BioLegend, San Diego, CA) or alkaline phosphate (AP; Bio-Rad) in TBST will be applied for one hour, followed by 3 washes and detection of HRP using enhanced chemiluminescence or alkaline phosphatase using 5-bromo-4-chloro-3-indolyl-phosphate/nitro blue tetrazolium (BCIP/NBT; Promega). Blots will be probed for PARP1, using PARP1 mouse monoclonal antibody (1:3000, C2-10, BD Biosciences) and DNMT1 and DNMT3a using mouse monoclonal antibodies (1:5000, Imgenex, San Diego, CA). Actin rabbit polyclonal (1:30000, Sigma-Aldrich) will be used as the loading control.

ii. **Q-Real-time-RT PCR:** mRNA will be isolated from AML MNCs (flash frozen) using the Machery-Nagel NucleoSpin RNA kit according to manufacturer's instructions. mRNA is then quatified on Nanodrop and diluted to 100-200ng/ul. cDNA is generated using the Applied Biosystems High Capacity cDNA RT kit. Preparation of the mastermix is performed according to manufacturer's instructions and mastermix and mRNA are added to a PCR tube or plate. Reverse transcription is performed in a BIORAD PCR machine with temperature and time settings according to instructions in the cDNA RT kit. cDNA is then diluted to 5ng per PCR reaction and reaction is set up in a 96- or 384-well plate using RT2 SYBR Green mastermix.. The plate is sealed and centrifuged. PCR is performed with a hot start of 90ºC for 10 min, and then 40 cycles of 95ºC for 15 seconds followed by 60ºC for 1 min in a BIORAD PCR machine.

iii. **Densitometry and Statistical Analysis:** Protein levels on immunoblots will be quantified by densitometry using the Quantity-one software (v.4.6, Bio-Rad) after scanning non-saturated luminograms. Band densities from treatment samples will be compared with band densities from pre-treatment samples and expressed as fold change. ***Result reporting:*** The log of the fold change relative to pre-treatment densities in three independent experiments will be reported as a mean value ± SEM. All statistical analyses will be performed using the Student t-test, with p-values ≤ 0.05 considered significant. We expect that protein levels of PARP1 and DNMTs will be significantly decreased in Day 5 post-treatment AML samples, compared with Day 1 pre-treatment samples. These results will be correlated with PARP trapping data in chromatin, using the Spearman's rank correlation.

9.5.7 Poly ADP Ribose (PAR) Polymerase Activity Assay

a) **Hypothesis and Rationale:** PAR activity should be decreased as a marker of talazoparib acitiviy.
b) **Intended Use Within this Study:** Decreased activity may correlate with the potential effects of the PARP inhibitor talazoparib
c) **Detailed Protocol:** The assay will be performed using the Trevigen HT PARP in vivo Pharmacodynamic Assay according to manufacturer's instructions (https://trevigen.com/docs/protocol/protocol_4520-096-K.pdf). In short, immobilized PAR monoclonal antibody in the wells of a 96-well plate capture cellular PAR and PAR attached to proteins. Incubation with a polyclonal PAR-detecting antibody, followed by addition of a goat anti-rabbit IgG-HRP secondary and a chemiluminescent HRP substrate yields relative light units (RLU) that directly correlate with the amount of cellular PAR.

9.5.8 DSB Repair

a) **Hypothesis and Rationale:** The combination of decitabine and talazoparib will lead to increased PARP trapping and decreased RAD51 foci formation that will decrease the HR DSB repair response, leading to increased cytotoxic DSBs. We will perform exploratory assays in AML participant samples to determine whether DSB NHEJ repair activity is altered in response to decitabine and talazoparib treatment.

b) **Intended Use Within this Study:** This study will determine whether levels of HR are decreased and whether levels of NHEJ are altered in response to treatment, in treated samples from AML participants (relative to pretreatment samples), as measured by HR and NHEJ assays, respectively, and whether these alterations will correlate with clinical responses.

c) **Detailed Protocol:** HR assay: *in vitro* HR assay will be performed using the HR assay kit (Norgen Biotek) according to kit protocol, with exceptions. Instead of transfecting AML MNCs, nuclear lysates from MNCs and HR plasmids (dl-1 and dl-2) will be incubated with nuclear lysates as described above. Following overnight incubation, plasmids repaired by HR will be assayed by RT-PCR (Bio-Rad) using the kit's assay and universal primers. NHEJ assay: Nuclear extracts will be isolated from AML MNCs (viably frozen), following standard procedures described previously (38,39). To measure efficiency of NHEJ, we will use a modified *in vitro* protocol of the EJ5-*Isce1* reporter assay as described in (40). The pimEJ5GFP reporter (Addgene, Cambridge MA) will be linearized with *I-Sce1* (New England Biolabs, Ipswich, MA) at 37^0C overnight, dephosphorylated using Shrimp Alkaline phosphatase (New England Biolabs) for one hour at 37^0C before column purification (Qiagen, Gaithersburg, MD). For the NHEJ reaction, 500ng of linearized EJ5-*Isce1* will be incubated with 5ug of E-buffer (20 mM Tris–HCl pH 8.0, 20% glycerol, 0.1 mM K(OAc), 0.5 mM EDTA, 1 mM DTT; 2 x 1hr) dialyzed nuclear extracts diluted in ligation buffer (10X T4 Ligase Buffer, 2 mM ATP, 50 µM deoxynucleotide triphosphates). After an overnight incubation at 16^0C, EJ5 will be purified through column purification and subjected to PCR for amplification of GFP genes using the primers P1 (Fwd) 5'-CTGCTAACCATGTTCATGCC-3', p2 (Rev) 5'-AAGTCGTGCTGCTTCATGTG-3', as described by (40). The PCR products is then run onto 2% Agarose gel and product will be visualized with the GelStar™ Nucleic Acid Stain (Lonza).

d) **Analysis and Result Reporting:** HR activity will be assessed using ΔΔCt method. Result reporting: The fold change in PCR product band density of treatment samples (C1D5 and C1D8) is estimated relative to that of C1D1 samples (pretreatment) using Bio-Rad's Quantity One Software. Log transformed fold changes will be compared between groups whenever relevant, as described above. For HR assay, samples will be normalized to C1D1 (set at 1) and expressed as fold change in HR activity. For NHEJ, ligated plasmid (PCR product) is calculated relative to total DNA loaded and expressed as relative efficiency of NHEJ repair (fold change compared to control). H3 expression is used as a loading control for the amount of protein in each reaction.

9.5.9 Immune Response Endpoint

Viably frozen cells and serum will be sent to VARI and these studies will be performed in the

future if funding allows. T-cell response pre- and post-treatment and cytokine release will be assessed.

9.6 DNA Methylation

Samples will be shipped to the VARI, where they will be processed and distributed for the studies, which will be performed in core laboratories of VARI-Stand Up to Cancer (SU2C) Epigenetics Dream Team members or collaborators.

Changes in DNA methylation will be studied, as follows:

a) Whole Genome Methylation Analyses Using Available DNA Methylation Array Platform, Such as the Illumnia Infinium Methylation Assay Platform (41).

The HumanMethylation450 BeadChip is a powerful tool in epigenetics research. The Illumina Infinium Methylation Assay uses only 1 µg of DNA and interrogates more than 485,000 methylation sites per sample at the single-nucleotide resolution. The array covers 99% of RefSeq genes, with an average of 17 CpG sites per gene region distributed across the promoter, 5'UTR, first exon, gene body, and 3'UTR. It covers 96% of CpG islands, with additional coverage in island shores and the regions flanking them.
Additional categories includes:

- CpG sites outside of CpG islands
- Non-CpG methylated sites identified in human stem cells
- Differentially methylated sites identified in tumor versus normal (multiple forms of cancer) and across several tissue types
- CpG islands outside of coding regions
- miRNA promoter regions

One 6.0 ml EDTA tube of bone marrow will be collected in a de-identified manner and shipped to VARI where the sample will be processed to remove WBC/Blast cell population for isolation of DNA that will be used in the 450K methylation array assays. VARI will ship a bone marrow (BM) specimen collection kit to the clinical study team. The BM specimen kit will have labels with a de-identified number set. The clinical study team will use the BM kit de-identified number to record for their participant records so that future 450K Infinium array research results can be correlated to the participants in the study.

The VARI Genomic Core Laboratory or VARI-SU2C collaborator will perform the methylation array according to standard operating procedures for sample processing, DNA isolation, and the methylation assay.

b) Bisulfite-Pyrosequencing of Line 1 + 10 Tumor Suppressor Genes (42).

DNA will be extracted and treated with bisulfite. Genomic DNA (2 µg) will be denatured by 0.2 M NaOH at 37°C for 10 minutes followed by incubation with freshly prepared 30 µL of 10 mM hydroquinone and 520 µL of 3 M sodium bisulfite (pH 5.0) at 50°C for 16 hours. DNA will be purified with a Wizard Miniprep Column (Promega, Madison, WI), desulfonated with 0.3 M NaOH at 25°C for 5 minutes, precipitated with ammonium acetate and ethanol, and dissolved in 50 µL of TE buffer (Tris-HCl 10 mM, EDTA 1 mM, pH 8.0). Bisulfite-treated DNA (40-80 ng) will be amplified with gene-specific primers in a 2-step polymerase chain reaction (PCR). The second

step of PCR will be used to label single DNA strand with biotin using a universal primer tag or gene-specific primers biotinylated at the 5′ end. Each PCR step will be performed in a total volume of 20 µL of 67 mM Tris-HCl (pH = 8.8), 16 mM ammonium sulfate, 2 mM $MgCl_2$, 0.125 mM dNTPs, 1 U *Taq* polymerase, and 100 nM PCR primers. TQ30 oligonucleotide (50 nM) will be used as a reversible inhibitor of *Taq* polymerase in the first step of the PCR.

DNA methylation will be measured as the percentage of bisulfite-resistant cytosines at CpG sites by pyrosequencing with the PSQ HS 96 Pyrosequencing System (Biotage, Charlottesville, VA) and Pyro Gold CDT Reagents (Biotage). Mean values from all pyrosequenced CpG sites will be used as a measure of methylation of a given gene. We will also measure DNA methylation at the 5′ end of the LINE-1 repeat (GenBank accession number X58075) to assess global methylation levels.

9.7 Potential Predictors of Response

Potential predictors of response will be explored (see section 13.6), including karyotype, *FLT3* and *NPM1* mutations, mutations in genes regulating methylation including *DNMT3A*, *TET2*, *IDH1*, *IDH2* and *EZH2*, as well as *PARP* expression levels. These studies will be performed on pre-treatment bone marrow, or on blood if marrow is inaspirable. Karyotype will be determined by standard cytogenetic analysis at each institution. Mutations will be studied as part a myeloid gene mutation panel at VARI. *PARP* expression levels will be measured as detailed above.

10. STUDY CALENDAR

Table 8. Study Calendar

	Pre-Study	Wk 1[h]	Wk 2[h]	Day 10[i]	Wk 3[h]	Wk 4[h]	Wk 5[h]	Wk 6[h]	Wk 7[h]	Wk 8[h]	Wk 9[h]	Wk 10[h]	Wk 11[h]	Wk 12[h]	…[h]	Off Study	Post-Study Follow-Up
Decitabine		A					A				A				A		
Talazoparib		B	B		B	B	B	B	B	B	B	B	B	B	B		
Informed consent	X																
Demographics	X																
Medical history	X	X	X		X	X	X	(X)	X	(X)	X	(X)	(X)	(X)		X	
Concurrent meds[f]	X		X----------												X		
Physical exam	X	X	X		X	X	X	(X)	X	(X)	X	(X)	(X)	(X)	X	X	
Vital signs	X	X	X		X	X	X	(X)	X	(X)	X	(X)	(X)	(X)	X	X	
Height	X																
Weight	X	X	X		X	X	X	(X)	X	(X)	X	(X)	(X)	(X)	X	X	
Performance status	X	X	X		X	X	X	(X)	X	(X)	X	(X)	(X)	(X)	X	X	
CBC w/diff, plts	X	X	X		X	X	X	(X)	X	(X)	X	(X)	(X)	(X)	X	X	
Serum chemistry[a]	X	X	X		X	X	X	(X)	X	(X)	X	(X)	(X)	(X)	X	X	
Coagulation studies	(X)																
EKG and/or Echocardogram	(X)																
Adverse event evaluation			X----------X												X	X	X
Survival Status																	X
B-HCG	X[b]																
Bone marrow aspirate, biopsy[c]	X						(X)				(X)				(X)		
Bone Marrow Cytogenetics	X																
Myeloid Mutation Panel[g]	X																
Buccal swab	X																
Blood correlative studies		X[d]		X[i]			X[e]				X[e]				X[e]		

A Decitabine IV daily, Days 1 through 5 or 10, at a consistent time, after talazoparib.
B Talazoparib orally daily continuously, taken at a consistent time in the morning.
a Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, magnesium, total protein, SGOT [AST], SGPT [ALT], sodium, uric acid.
b Serum pregnancy test (women of childbearing potential).
c Bone marrow aspirate and biopsy will be performed on day 25-29 of cycle 1, as well as on day 25-29 of each treatment cycle (unless circulating blasts persist in the peripheral blood), until documentation of CR or CRi. Bone marrow aspirate and biopsy will also be performed as needed to evaluate lack of count recovery (neutrophils <1000/μL and/or platelets <50,000/μ) at week 7 in the absence of blood blasts, and at time of clinical concern for disease progression after CR or CRi.
d Blood pharmacodynamic samples (green and lavender tubes) in Cycle 1 on Day 1 pre-treatment, on Day 5 1-hour post decitabine treatment, and on Day 8. For participants receiving 10-day decitabine, sampling will be on Days 1, 5, 8 and 10, one hour after the decitabine injection.
e Blood pharmacodynamic samples (lavender tubes only) in subsequent Cycles on Day 29.
f Any medications, including supplements and herbs
g At the discretion of the treating Physician
h (± 3 days) i for Cohort 7 and/or the expanded cohort only
( ) As clinically indicated, if needed, throughout the study.

10.1 Screening Evaluations

Baseline evaluations are to be conducted after patients sign informed consent and within 14 days prior to start of protocol therapy. In the event that the participant's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of therapy.

Screening evaluations include:

- Medical history and physical examination, including measurements of height and weight, performance status, vital signs (blood pressure, pulse rate, respiration rate and temperature); concurrent medication assessment
- Complete blood count (CBC) (hemoglobin, WBC, and platelet count) and manual differential white blood cell count;
- Coagulation studies: aPTT, PT, INR, fibrinogen, with D-dimer as clinically indicated;
- Blood chemistries: electrolytes (sodium, potassium, bicarbonate and chloride), glucose, BUN, creatinine, ALT, AST, total bilirubin, alkaline phosphatase, calcium, phosphorus, magnesium, albumin, total protein, LDH and uric acid;
- Blood pregnancy test for women of childbearing potential;
- Bone marrow aspirate and biopsy
- Bone marrow cytogenetics
- Bone marrow myeloid mutation panel (at discretion of treating Physician)
- Two Buccal swabs for mutation studies
- Electrocardiogram (EKG) and/or echocardiogram (ECHO) will be performed only as clinically indicated.

10.2 Study Evaluations

10.2.1 Cycle 1

- During treatment cycle 1 on study, history and physical examination with vital signs, weight and performance status should be performed at least weekly and can be performed on any day of the week.
- CBC/manual differential and comprehensive chemistry tests should be performed at least weekly.
- Blood must be drawn for pharmacodynamics and research testing on day 1 before treatment, on day 5 one hour after decitabine injection and on day 8 at any time.
- For participants receiving 10-day decitabine, sampling will be on Days 1, 5, 8 and 10, one hour after the decitabine injection.
    - The Cycle 1 Day 10 visit will coincide with an infusion visit and will consist of a correlative blood draw only and can be handled as a nurse visit.
- Coagulation studies should be performed as clinically indicated.
- Bone marrow aspirate and biopsy should be performed on day 25-29 of the first treatment cycle.
- Toxicity rating using the NCI Common Terminology Criteria for Adverse Events version 4.0 will be performed as needed.

10.2.2 Cycle 2

- During treatment cycle 2, physical examination with vital signs, medical history, weight and performance status should be performed at least every two weeks and can be performed on any day of the week.
- CBC/manual differential and comprehensive chemistry tests should be performed at least every two weeks.
- Coagulation studies should be performed as clinically indicated.
- Bone marrow aspirate and biopsy should be performed pre-dose, approximately on day 25-29 unless circulating blasts persist in the peripheral blood.
- Toxicity rating using the NCI Common Terminology Criteria for Adverse Events version 4.0 will be performed as needed.

10.2.3 Subsequent Cycles

- During cycle 3 and subsequent cycles, physical examination with vital signs, weight, and performance status should be performed at least on day 1 of each treatment cycle.
- CBC/manual differential and comprehensive chemistry tests should be performed at least on day 1 of each treatment cycle.
- Coagulation studies should be performed as clinically indicated.
- Bone marrow aspirate and biopsy should be performed pre-dose, approximately on day 25-29 of each treatment cycle unless circulating blasts persist in the peripheral blood, until documentation of CR or CRi. Bone marrow aspirate and biopsy will also be performed pre-dose, as needed to evaluate lack of count recovery (neutrophils <1000/μL and/or platelets <50,000/μL) by day 43 in the absence of blood blasts, and at time of clinical concern for disease progression after CR or CRi.
- EKG and/or ECHO will be performed only as clinically indicated.
- Toxicity rating using the NCI Common Terminology Criteria for Adverse Events version 4.0 will be performed as needed.

10.2.4 Off Study Assessments

Once a participant discontinues participation in the study, the participant will be asked to return within 30 days for the following assessments. This return visit will depend upon the status of the participant and while recommended, is not required:

- Performance status evaluation
- Limited medical history and physical examination
- Vital signs, weight
- CBC with manual
- Differential, platelet count
- Blood chemistries
- Adverse event evaluation

10.3 Post-Study Follow-up

Participants will be followed for survival and possible long-term toxicity from treatment. Follow-up will continue at least every 6 months until death. If not seen at hospital visits, participants will be

contacted by telephone or by email. Further therapy will be at the discretion of the treating physician.

11. MEASUREMENT OF EFFECT

Response definitions will be according to the revised *International Working Group (IWG) response criteria for AML* as published by Cheson et al. (43).

11.1 Definitions of Response for Acute Leukemia

11.1.1 Complete Remission (CR):

Bone marrow with < 5% blasts and with maturation of all cell lineages, no Auer rods, an absolute neutrophil count ≥ 1,000/μl and a platelet count ≥ 100,000/μl, transfusion independence, absence of blasts in peripheral blood, absence of identifiable leukemic cells in the bone marrow, clearance of any extramedullary disease (soft tissue involvement). Clearance of cytogenetic abnormalities will not be required for CR, but will be noted and described separately.

11.1.2 Complete Remission with Incomplete Blood Count Recovery (CRi):

CRi satisfies all CR criteria except for residual neutropenia ANC < 1,000/μl and/or platelets < 100,000/μl, but platelet transfusions are not necessary.

11.1.3 Partial Remission (PR):

Must meet all criteria of a CR except that the bone marrow may contain 5-25% blasts, and at least 50% decrease in bone marrow blast percentage from baseline must be present.

11.1.4 Hematologic Improvement (HI):

At least 50% decrease in marrow blasts or extramedullary disease, including peripheral blood blasts; ANC 500-1000/μl; platelets 20,000-100,000/μl or improved transfusion needs.

11.1.5 No Response (NR):

No criteria for CR, CRi, PR or HI are met.

11.1.6 Relapse:

Any of the following, occurring after either CR, CRi, or PR:

- The reappearance of circulating blasts not attributable to “overshoot” following recovery from myelosuppressive chemotherapy (at least 2 measurements).
- ≥ 5% blasts in the bone marrow, not attributable to another cause (e.g. bone marrow regeneration), or in case of PR > 50% increase in bone marrow blast % from best assessment.
- The reappearance or development of cytologicaly proven extramedullary disease.

- Molecular and/or genetic relapse is characterized by reappearance of a cytogenetic or molecular abnormality.

11.1.7 Duration of Response

The duration of response is measured from the time measurement criteria for CR or CRi are met until the first date that recurrent or progressive disease is objectively documented. The duration of CR is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented

11.1.8 Progression-Free Survival

All participants will be assessed for PFS, defined as the time between study entry and the first date that recurrent or progressive disease is objectively documented, or death from any cause occurs.

11.1.9 Overall Survival

Overall survival will be measured from time of enrollment onto this study to the time of death.

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in *Section 7* (Adverse Events: List and Reporting Requirements).

12.1 Compliance with Laws and Guidances

This study will be conducted in accordance with current U.S. Food and Drug Administration (FDA) Regulations, Good Clinical Practices (GCPs) and International Counsel on Harmonization (ICH) E6 Guidelines.

12.1.1 Institutional Review Board (IRB)

This protocol must be submitted to a local IRB in accordance with the Code of Federal Regulations (CFR), and FDA and International Counsel on Harmonization (ICH) E6 Guidelines. Study procedures will begin once local IRB approval is secured. All amendments, instances of reportable new information (i.e. unanticipated problems, data breaches, etc.) and continuing review reports must be submitted to the local IRB of record.

12.1.2 Clinical Trial Monitoring

Data safety and verification monitoring will be conducted in accordance with the Greenebaum Comprehensive Cancer Center Sponsor-Investigator Monitoring Standard Operating Procedure (SOP), the Code of Federal Regulations (CFR), and FDA and International Counsel on Harmonization (ICH) E6 Guidelines.

12.1.3 Data and Safety Monitoring Board (DSMB)

DSMB oversight will be conducted in accordance with the Greenebaum Comprehensive Cancer Center DSMB standard operating procedures.

It is the responsibility of the Principal Investigator to oversee the safety of the study. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above.

This study will be monitored by the UMGCCC Data and Safety Monitoring and Quality Assurance Committee and will follow the Data Safety and Monitoring Plan as outlined in the Clinical Investigator Handbook of the UMGCCC Clinical Research Office. Monitoring will be conducted as per the plan on file with the University of Maryland IRB (also available in the UMGCCC Clinical Investigator Handbook found at http://www.umgcc.org/research/clinical_research.htm).

12.1.4 FDA Reporting

The FDA Annual Report will be submitted to the Agency within +/- 60 days of the date on the IND approval letter. Major amendments will be submitted to FDA at the time of IRB submission. Minor amendments will be reported to FDA at the time of the next major amendment or at the next FDA Annual Report (whichever comes first).

12.1.5 Records Retention

Essential documents should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. Records and documents pertaining to the conduct of this study and the distribution of investigational drug, including CRFs, consent forms, laboratory test results, and medication inventory records will be maintained in secure offsite storage after completion of study follow-up and data analysis.

12.1.6 Compliance with Trial Registration and Results Posting Requirements

Under the terms of the Food and Drug Administration Modernization Act (FDAMA) and the Food and Drug Administration Amendments Act (FDAAA), the Sponsor of the trial is solely responsible for determining whether the trial and its results are subject to the requirements for submission to the Clinical Trials Data Bank, http://www.clinicaltrials.gov. Information posted will allow participants to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and trial site contact information.

12.1.7 University of Maryland Data Management

Clinical data will be entered into the OnCore® database by the designated data manager at the University of Maryland Marlene and Stewart Greenebaum Comprehensive Cancer Center (UMGCCC). Oncore® is used for data entry in a way that is compliant with 21CFR11.10 (electronic medical records) and is equipped for HIPAA-compliant internet-based entry of protocol tracking and review information.

All study data will be collected by the research team at each and every study visit and recorded

in the research record. This data will then be entered in to the OnCore® study database.

All source documents will be obtained and retained along with any study forms, and placed into the participant's research folder.

12.2 Multi-institutional Data Management

Remote VPN access will be provided for each participating site along with Oncore® training. This access and training will occur prior to enrollment of the first participant at the site, in order to ensure that staff are adequately trained and able to enter data within 5 days of the visit occurring. All study data will be collected by the research team at each and every study visit and recorded in the research record. This data will then be entered in to the OnCore® study database.

All source documents will be obtained and retained along with any study forms, and placed into the participant's research folder according to the policies around record retention in place at each site.

13. STATISTICAL CONSIDERATIONS

13.1 Dose Escalation and Nesting of Dose Levels

The statistical design of this Phase 1 trial is a 'traditional' 3+3 design. Only participants with relapsed or refractory AML will be enrolled in the trial. Participants who withdraw from the trial for any other reason than toxicity and who have not already developed a dose-limiting toxicity (DLT) at that point, will be replaced by recruiting another participant at the same dose level. Due to the clinical setting and the putative mechanistic action of talazoparib as a sensitizer of the effect of decitabine, escalation of the two agents will use a nested Phase I design giving priority to the dose of decitabine: the design will estimate the maximum tolerable dose of decitabine in combination with a dose of talazoparib of 0.25 mg or higher. The nested design is defined by Table 9 below:

Table 9 Dose Escalation and Nesting of Dose Levels

		Talazoparib (mg)				
		0.25	0.50	0.75	1.0	1.0
Decitabine (mg/m^2)	10	1	1a	1b	1c	-
	15	2	2a	2b	2c	-
	20	3	4	5	6	7*

* Cohort 7 will receive 20 mg/m2 of decitabine for 10 days and 1 mg of Talazoparib continuously.

The 'outer layer' of this nested dose escalation trial will escalate the dose of the two drugs by sequentially going through dose levels 1-6 in the table. The standard algorithm of the 3+3 design will be applied as specified below.

If Cohort 6, with 20 mg/m^2 decitabine daily for 5 days and talazoparib 1 mg daily, is completed without dose-limiting toxicity, Cohort 7, consisting of decitabine, 20 mg/m^2 daily for 10 days and talazoparib, 1 mg daily, will be added.

Following completion of Cohort 6 or Cohort 7 without dose-limiting toxicity, a maximum of 17 participants not previously treated with DNMTis (decitabine, azacitidine and/or guadecitabine) will be enrolled to assess response in this patient population.

If the MTD has not been reached at dose level 7, the RP2D of decitabine will be 20 mg/m^2 for 10 days combined with a dose of talazoparib of 1.0 mg.

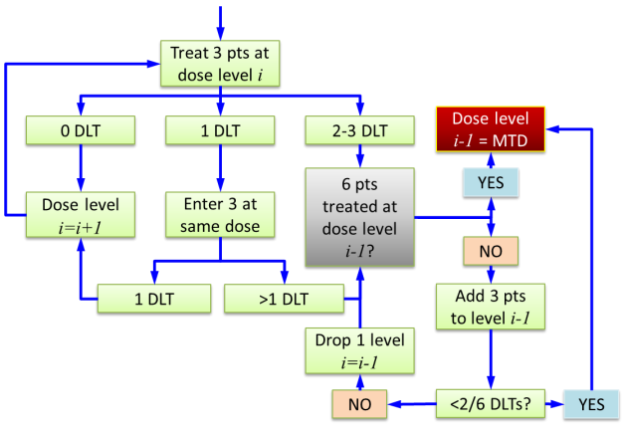
At the time of revising the protocol, the toxicity data demonstrated that the MTD has not been reached at dose level 5. The following decision rules were not activated but are preserved in this version of the protocol for completeness.

If 2 or more participants develop DLT at dose level 1, the combination of decitabine and talazoparib will be considered too toxic for further clinical development.

If the *provisional* MTD for decitabine in combination with talazoparib is dose level 1 or 2, one or the other of the 'inner layers' of the trial will be activated. If the provisional MTD corresponds to level 2, the dose of decitabine will be fixed at 15 mg/m^2 and the dose of talazoparib will be escalated through steps 2a-c, again using the algorithm of the 3+3 design below. If the tolerance is not exceeded at level 2c, then the RP2D of decitabine is 15 mg/m^2 combined with a dose of talazoparib of 1.0 mg.

Similarly, if the provisional MTD for the combination of the two agents from the outer layer is level 1, the dose of decitabine will be fixed at 10 mg/m^2 and the dose of talazoparib will be escalated through steps 1a-c, again using the algorithm of the 3+3 design below to define the MTD of the combination.

13.2 '3+3' Design



There are several variations to the 3+3 design, and it may therefore be helpful to describe the current design in detail. Three participants will be accrued at each dose level until a DLT is seen, see flow diagram. The first dose level at which one DLT is seen will be expanded to six participants. If only one DLT is seen, dose escalation will continue in three-participant cohorts. If one DLT is seen among the three participants accrued at one of the subsequent dose levels, the cohort treated at that dose level will be expanded to six participants. If two or more of the six participants experience DLT, then the maximum tolerated dose (MTD) is considered to be exceeded, and the next lower dose level is considered the MTD provided that 6 participants were treated at this dose level. If not, this level is considered a candidate MTD and expanded to 6 participants. The algorithm will stop when there is <2 of 6 participants with DLTs at the candidate MTD level and this will be defined as the MTD.

The minimum number of participants in the trial is 2 – this would be the number enrolled if the two first participants at level 1 both were to experience a DLT. The maximum number of dose combinations tested is 7 This yields a – hypothetical – upper limit on the number of participants

enrolled of 59, arising if exactly 1 DLT is observed at each of the 7 dose levels plus an additional 17 patients for the post Cohort 6 / 7 DNMTi-naïve expansion cohort.

If Cohort 6, with 20 mg/m^2 decitabine daily for 5 days and talazoparib 1 mg daily, is completed without dose-limiting toxicity, Cohort 7, consisting of decitabine, 20 mg/m^2 daily for 10 days and talazoparib, 1 mg daily, will be added.

Following completion of Cohort 6 or Cohort 7 without dose-limiting toxicity, a maximum of 17 participants not previously treated with DNMTis (decitabine, azacitidine and/or guadecitabine) will be enrolled to assess response in this patient population.

The aim of the expansion cohort will be to expand the safety data and to look for signs of activity in the DNMTi-naïve population.

13.2.1 Recommended Phase 2 Dose (RP2D)

The MTD of the combination of decitabine with a dose of talazoparib of at least 0.25 mg is defined as the maximal tolerated dose of decitabine studied – and for that dose level, combined with the maximum dose of talazoparib for which the incidence of DLT was less than 33% in 6 participants treated and this will be chosen as the recommended Phase 2 dose (RP2D).

Among potential combined dose levels at MTD, available pharmacodynamic data (PARP trapping) will also be considered in the choice of RP2D, as will any significant indications of differences in clinical efficacy.

13.3 Expansion cohort at RP2D

Testing of decitabine combined with talazoparib at the RP2D will be conducted with Overall Complete Remission (OCR) as the primary endpoint in an expansion cohort with the aims of expanding the safety data and looking for signs of activity in the DNMTi-naïve population. If at least three of 17 DNMTi-naïve patients respond to treatment, we will proceed with the Phase 2 trial.

13.4 Phase 2

Trial A will enroll adult participants with AML who are deemed unfit for standard cytotoxic chemotherapy;

Trial B will enroll adult participants with relapsed or refractory AML;

The phase II component of the trial will use Simon's optimal two-stage design (44) with a 1-sided α=0.05 and power (1-β) of 80%. This design specifies two target response probabilities: p_0 is the OCR corresponding to the null hypothesis that the combination of the two drugs is not more active than decitabine alone; p_1 is the OCR considered "promising" for further development of the combination in a future trial.

Staggered entry of participants will be allowed due to the relatively long delay between enrolling a participant and verification of an OCR and the expected relatively high accrual rate from the

multi-centric collaborative network. A maximum of 12 participants will be allowed to be enrolled in each trial in addition to the number required in the first stage of the trial before making the call regarding possible closure of the trial due to futility. Staggered entry is considered acceptable as decitabine is an active standard therapy in the studied indications and there is no mechanistic reason to expect an antagonistic interaction from adding talazoparib.

13.3.1 Trial A (unfit for chemotherapy, azacitidine-, decitabine- and guadecitabine-naïve)

We select a p_0=25% response rate as the null hypothesis for the OCR after treatment with the combination of decitabine and talazoparib. For p_1 we choose 40%.

The null hypothesis, that the true OCR is 25%, will be tested against the 1-sided alternative hypothesis. In the first of the two stages of the trial, 25 participants will be enrolled. If 6 or fewer responses are achieved in these 25 participants, the study will be stopped early for futility. Otherwise, an additional 38 participants will be enrolled. The null hypothesis will be rejected if 22 participants or more achieve a response out of the total 63 participants enrolled. This design yields a type I error rate (α) of 0.047 and a power of 80.0% when the true response probability is 40%.

13.3.2 Trial B (relapsed or refractory, azacitidine-, decitabine- and guadecitabine-naïve)

The null hypothesis, that the true response probability is p_0=10%, will be tested against the 1-sided alternative hypothesis, p_1=25%. In the first stage of the phase II trial, 22 participants will be enrolled. If 2 or fewer responses are achieved in these 22 participants, the study will be stopped early for futility. Otherwise, an additional 18 participants will be enrolled. The null hypothesis will be rejected if 8 or more participants achieve a response out of the total 40 participants enrolled. This design yields a type I error rate (α) of 0.040 and a power of 80.03% when the true response probability is 25%.

A minimum of 25 participants and a maximum of 63 participants will be enrolled in Trial A of Phase II. A minimum of 22 participants and a maximum of 40 participants will be enrolled in Trial B of Phase II.

In Phase II of this study a total minimum of 44 participants and a total maximum of 103 participants will be enrolled.

13.4 Stratification Factors

There is no stratification in phase I of this trial. Stratification in phase II is based on disease status and prior treatment and is effectuated by allocation to one of trials A or B that will be run in parallel and each will be analyzed and reported separately.

13.5 Analysis of Secondary Endpoints

In Phase I, we will tabulate the incidence of OCR as a function of dose level. These data will be subject to a binary logistic regression model to test for a trend in OCR as a function of the dose of each of the two agents.

A more detailed characterization of the safety profile of the drug combination delivered at the

RP2D will be reported from the phase II population. The distribution of the grade of toxicity according to the CTCAE ver. 4.0 as well as the incidence of high grade toxicities with exact 95% CIs will be reported. In case of transient toxicities, the Kaplan-Meier estimate of the time to resolution will be presented.

13.6 Biomarkers and Pharmacodynamic Markers

An exploratory, hypothesis-generating analysis of heterogeneity of treatment effects in will be conducted using binary logistic regression with OCR as the dependent variable. Participant-related factors as well as pharmacodynamic and biomarker data will be tested as explanatory variables. The results of this analysis will be reported in addition to the main trial outcomes. Potential covariates to be tested include karyotype, FLT3 and NPM1 mutations, mutations in genes regulating methylation including DNMT3A, TET2, IDH1, IDH2 and EZH2, as well as PARP expression levels and participant and treatment characteristics. In addition, ordinal logistic regression will be conducted with the actual response level as the dependent variable and pharmacodynamic and biomarker data as explanatory variables, as above.

13.7 Reporting and Exclusions

All participants who received at least one treatment cycle will be included in the intention-to-treat (ITT) population and this population will be the basis for the primary analysis and reporting of efficacy and toxicity outcomes in the three parallel phase II trials. For each trial, the proportion of participants who achieved OCR with exact 95% CIs will be reported as will the distributions of relapse-free survival, overall survival, and 100 day mortality using Kaplan-Meier estimates.

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APPENDIX A –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 B – AML SAMPLE PROCUREMENT

PURPOSE

This procurement procedure describes the processes for collecting and shipping samples for the Van Andel Research Institute-Stand Up 2 Cancer (VARI-SU2C) Epigenetics Dream Team Project entitled, *Multicenter Phase 1/2 Study of Combination Therapy with the DNA Methyltransferase Inhibitor Decitabine and the Poly ADP Ribose Polymerase (PARP) Inhibitor Talazoparib (BMN 673) for Acute Myeloid Leukemia (AML) in Adult Patients with Relapsed/Refractory Disease* (Short name: AML Project).This includes collection supplies and kits, collection forms, consent, collection guidelines and shipping instructions.

SAFETY

All study personnel involved in this procedure are required to follow their institutional safety procedures, including, but not limited to, appropriate personal protective equipment such as gloves, lab coats and protective glasses. Treat all tissue and blood products as potentially infectious and dispose of them according to site institutional procedures and protocols.

SUPPLIES

- EDTA Tubes (Preferred: BD Vacutainer® 6.0 mL lavender-top EDTA Blood Collection tubes, supplied by clinical site)
- Sodium Heparin collection tubes (Preferred: BD vacutainer ® 6.0 mL green-top tubes, supplied by clinical site)
- Buccal swab kit (supplied by VARI)
- Specimen shipping kit (supplied by VARI)

PROCEDURE

1. <u>Collection Supplies</u>

1.1. VARI will supply each collection site with a stock of buccal swab kits and blank sample labels to be affixed to each sample.

1.2. The clinical site will supply EDTA (lavender-top) and Sodium Heparin (green-top) collection tubes.

1.3. VARI will provide each site with kits to return the samples to VARI. These kits will contain, at minimum, the following:

1.3.1. Insulated shipper

1.3.2. 2 cold packs

a. Remove both cold packs and freeze at -20°C for at least 2 hours. Cold packs should remain frozen until shipment.

1.3.3. Pre-printed return label (Fed-Ex)

2. Data Collection Form

2.1. All tissue collected must be accompanied by a completed VARI-SU2C *Data Collection Form*. The *Data Collection Form* must be completed prior to shipment of samples.

2.2. The *Data Collection Form* is specific to a Participant collection event. The following fields should be completed:

2.2.1. De-Identified Study Participant ID: This is a de-identified study participant identifier maintained only by the clinical site performing the collection. This identifier must be linked to the medical record number of the study participant.

a. This number must be assigned with each collection and must be compared to the master list to ensure all participants only receive one de-identified participant ID.

2.2.2. Collection Date: The date the tissue was collected and prepared.

2.2.3. Collection Time: The time of both blood and bone marrow collection.

2.2.4. Timepoint: The collection event timepoint. This will be protocol specific, such as Pre-treatment, cycle 1, Day 8, etc.

2.2.5. Form Completed By: The person filling out the data collection form.

2.2.6. Age: The age of the participant at time of tissue/blood collection

2.2.7. Sex: The sex of the participant.

2.2.8. Race/Ethnicity: The race and/or ethnicity of the participant recorded in collection (clinical) site's electronic medical record.

2.2.9. Number of EDTA (blood) tubes submitted.

2.2.10. Number of Sodium Heparin (bone marrow) tubes submitted.

2.2.11. Check whether the bone marrow is in good condition and not clotted.

2.2.12. Circle the aspirate number of the bone marrow submitted.

2.2.13. Circle yes or no to indicate whether the bone marrow was collected in a heparin containing syringe.

2.2.14. Person responsible for sample shipment.

2.2.15. Person completing the form.

2.2.16. Any additional comments or notes important to the collection/shipping event.

3. Participant Consent

3.1. All participants must consent for any donation prior to any specimen collection. Please refer to the collection site's Trial Study consent protocols for all consent procedures.

4. Blood/Bone Marrow/Saliva Procurement Guidelines

4.1. Peripheral Blood Collection

4.1.1. See Section 9 of this protocol for blood collection timepoints.

4.1.2. Collect 10mL of blood in two 6mL lavender-top, K2-EDTA vacutainers. If 6mL lavender K2-EDTA tubes are not available, collect 10mL of peripheral blood in available size lavender-top, K2-EDTA tubes.

a. Draw peripheral blood according to collection site's institutional venipuncture protocols.

4.1.3. Collect 20mL of blood in four 6mL green-top, sodium heparin (NaHep) vacutainers. If 6mL NaHep tubes are not available, collect 20mL of peripheral blood in available size green-top (NaHep) tubes.

4.1.4. Invert each vacutainer (green and lavender) 8-10 times to ensure sufficient mixing.

4.1.5. Label each tube with the De-identified Participant Study ID, participant initials, date (of collection), timepoint (cycle/day) and sample type (PB).

4.1.6. Keep samples at room temperature.

4.1.7. Process samples within one hour of collection.

4.2. Bone Marrow Collection

4.2.1. See Biomarker and Correlative Studies protocol section for bone marrow collection timepoints.

4.2.2. Collect 10mL of bone marrow in two 6mL green-top, Sodium Heparin (NaHep) vacutainers. If 6mL NaHep tubes are not available, collect 10mL of bone marrow in available green-top, NaHep tubes.

a. Draw bone marrow according to collection site's institutional bone marrow aspiration protocols.

4.2.3. Invert each vacutainer 8-10 times to ensure sufficient mixing.

4.2.4. Label each tube with the De-Identified Study Participant ID, participant initials, date (of collection), timepoint (cycle/day) and sample type (BM).

4.2.5. Keep samples at room temperature.

4.2.6. Process samples within one hour of collection.

4.3. Saliva Collection

4.3.1. Utilizing the Isohelix Buccal Swab kit, open the package from one end.

4.3.2. Remove the swab from the tube.

4.3.3. Insert the swab into the Participant's mouth and rub firmly against the inside of the cheek or underneath the lower or upper lip. Rub for 1 minute. Use reasonable, firm and solid pressure.

4.3.4. Place the swab back into the tube. Do not touch the brush with your fingers.

4.3.5. Place thumbnail in the small groove set in the handle, then snap the handle in two by bending to one side. Let the swab head fall into the tube.

4.3.6. Seal the tube securely with one of the caps.

4.3.7. Label each tube with the De-identified Participant Study ID, participant initials, date (of collection), timepoint (cycle/day) and sample type (swab).

5. Blood/Bone Marrow/Saliva Shipment

5.1. Ship all samples on the same day as collection.

5.2. Confirm *Data Collection Form* is completed.

5.3. Make a copy of the *Data Collection Form* and retain for your institution's study records.

5.4. Pack the shipping container:

5.4.1. Place all collection vacutainer tubes in the small, inner box. Remove and dismantle the styrofoam insert for tube placement. Each side of the styrofoam compartment in the smaller inner box holds 4 tubes.

a. Place the peripheral blood vacutainers in the 4 grooves in one side (ensure that the blood tubes have been properly mixed, 5-10 times, top to bottom, prior to shipment).

b. Place the bone marrow vacutainers in 2 grooves in the opposite side(ensure that the bone marrow tubes have been properly mixed, 5-10 times, top to bottom, prior to shipment).

c. Place the buccal swab tube into a groove on the same side and the bone marrow vacucatiners.

5.4.2. Place the fully assembled styrofoam insert into the provided Biohazard Ziploc bag with absorbent paper and seal the Ziploc bag.

5.4.3. Place the Ziploc bag with the Styrofoam insert inside the small cardboard box and close the box.

5.4.4. Place the small box inside the larger plastic ziplock bag then place it in the large, outer insulated shipper.

5.4.5. Retrieve the 2 frozen cold packs, ensure they are completely frozen, and place them on either side of the small, inner box.

5.4.6. Place the Styrofoam lid on the insulated shipper and place the completed *Data Collection Form* on top of the lid.

5.4.7. Close the cardboard flaps and seal the package. Be sure to remove the pre-printed FedEx air bill from the outer insulated shipper prior to sealing the package.

5.5. Peel off the yellow removable label covering the UN3373 sticker on the outside of the insulated shipper.

5.6. Once shipments are scheduled, send an email to pbs.lab@vai.org to notify VARI of incoming shipment. Please include the following information:

5.6.1. Subject line: "VARI-SU2C AML Project shipment from (*collection site*)"

5.6.2. Email body:

Ship date
FedEx tracking number (found on pre-printed air bill)
Collection site name and De-Identified Study Participant ID

5.6.3. Schedule each shipment with FedEx (1-800-463-3339) using local pick-up/drop-off locations.

a. If FedEx is unable to pick up the sample due to a late collection, refrigerate the samples until the next available FedEx pickup time.

For any additional questions or to request collection kits, contact VARI at 616-234-5122 or pbs.lab@vai.org.

APPENDIX C – PARTICIPANT DIARY FOR TALAZOPARIB

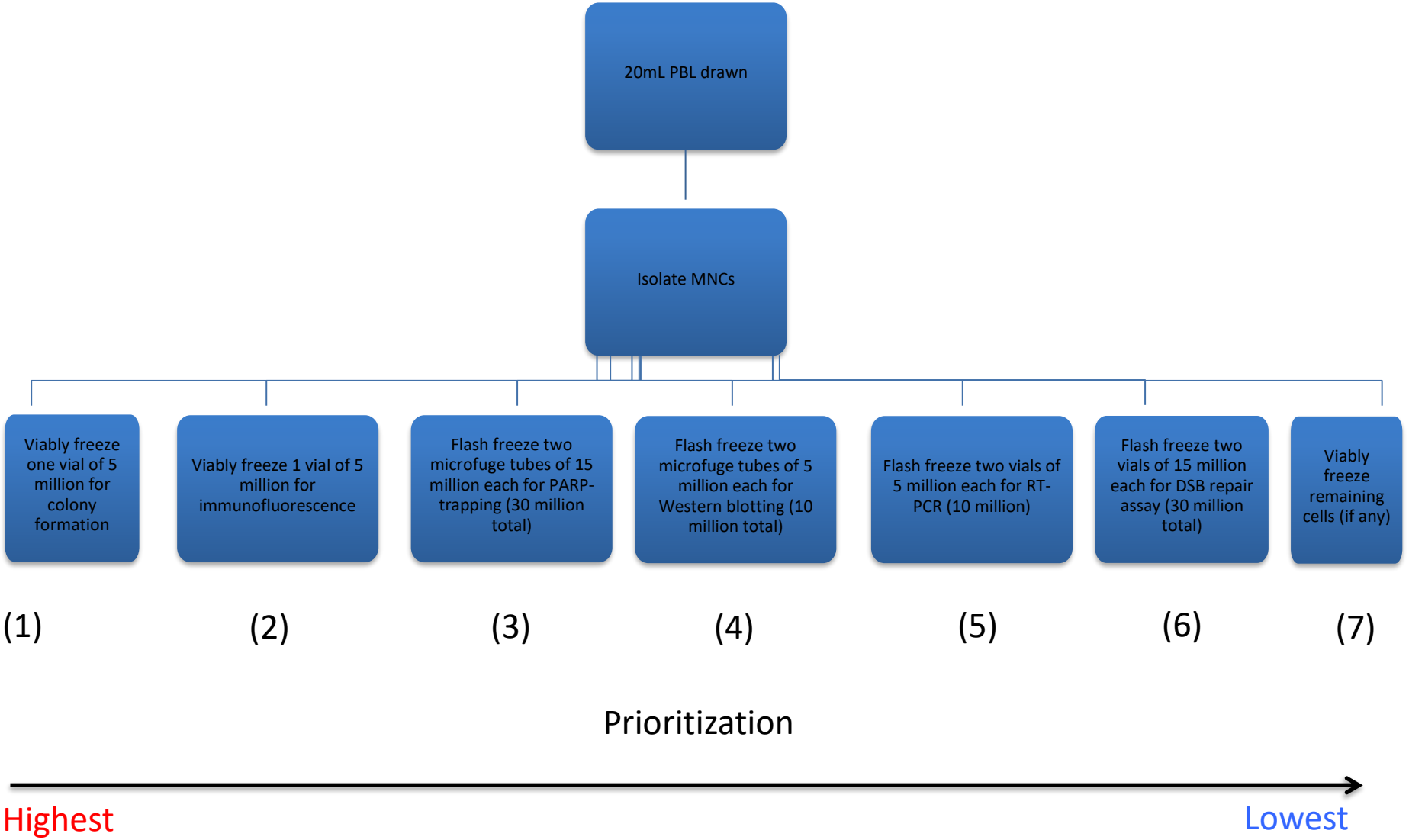
Note: Talazoparib should be taken at a consistent time every day. Please complete this diary each day after you take your dose of Talazoparib.

Day of Study	Date	Dose taken	Time taken	If not taken, reason
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				

Please remember to return this form and any unused Talazoparib to the study staff. Thanks!

To be Completed by Staff:
Received on _____ by _____

APPENDIX D – LAB ANALYSIS PRIORITY DIAGRAM



INVESTIGATOR AGREEMENT

University of Maryland Local Protocol #: 1565GCC

ClinicalTrials.gov Identifier: NCT02878785

Title: Multicenter phase 1/2 study of combination therapy with the DNA methyltransferase inhibitor decitabine and the poly ADP ribose polymerase (PARP) inhibitor talazoparib (BMN 673) for untreated acute myeloid leukemia (AML) in adult patients unfit for cytotoxic chemotherapy or relapsed/refractory AML

I have read this protocol in its entirety and agree that it contains all necessary details for carrying out the study. I will conduct this study as outlined and within the time periods designated. I understand that the study may be terminated or enrollment suspended at any time by the sponsor, with or without cause, or by me if it becomes necessary to protect the best interests of the study participants. I confirm that I will conduct this study in accordance with the protocol as written, and in accordance with ICH GCP guidelines, the ethical principles of the Declaration of Helsinki, and applicable local regulations.

I will provide copies of the protocol and all study related information to all responsible individuals who assist me in the conduct of this study. I will discuss this material with them to ensure they are fully informed of the conduct of the study, the study drug, and the obligations of confidentiality.

Principal (Site) Investigator:

Name (printed or typed): _____

Institution and Address:

_____

_____

_____

_____

Telephone Number: _____

Signature: _____ Date: _____