SUMMARY OF CHANGES – Protocol

For Protocol Revision 16 to: **BL**ockade of PD-1 **A**dded to **S**tandard **T**herapy to target **M**easurable **R**esidual **D**isease in **A**cute **M**yeloid **L**eukemia 2 (BLAST MRD AML-2): A randomized Phase 2 Study of the Venetoclax, Azacitidine, and Pembrolizumab (VAP) Versus Venetoclax and Azacitidine as First Line Therapy in Older Patients with Acute Myeloid Leukemia (AML) who are Ineligible or who Refuse Intensive Chemotherapy

NCI Protocol #: 10334
Local Protocol #: 2000031641

NCI Version Date: 11/27/2024
Protocol Date: 11/27/2024

I. Recommendations per Review of Amendment #10 by Brian Ko, MD dated 11/04/2024.

#	Section	Comments
1.	All	Updated Version Date in Header
2.	Title Page	Revised Protocol Type / Version# / Version Date
3.	Table of Contents	Updated Table of Contents
4.	5.7 5.9.6.2	Please specify "skin" punch biopsies. **PI Response: This has been updated.**
5.	5.9.9.1	Please make sure that the address for shipment of specimens for methylation assay is correct. Beatriz Sanchez-Espiridion's name was removed from other assays. **PI Response: Thank you for the comment. We are awaiting confirmation of the address but needed to update the study personnel right away. The address change will be provided once MD Anderson has confirmed.**
6.	13.5-13.6	Please delete both sections. **PI Response: These sections have been deleted.**

II. Additional Changes by Principal Investigator

#	Section	Comments
7.	Title Page	Added Jan Bewersdorf as Co-Principal Investigator
8.	All	Formatting, typos corrected throughout.

NCI Protocol #: 10334

Local Protocol #: 2000031641

ClinicalTrials.gov Identifier: NCT04284787

TITLE: BLockade of PD-1 **A**dded to **S**tandard **T**herapy to target **M**easurable **R**esidual **D**isease in **A**cute **M**yeloid **L**eukemia 2 (BLAST MRD AML-2): A randomized Phase 2 Study of the Venetoclax, Azacitidine, and Pembrolizumab (VAP) Versus Venetoclax and Azacitidine as First Line Therapy in Older Patients with Acute Myeloid Leukemia (AML) who are Ineligible or who Refuse Intensive Chemotherapy

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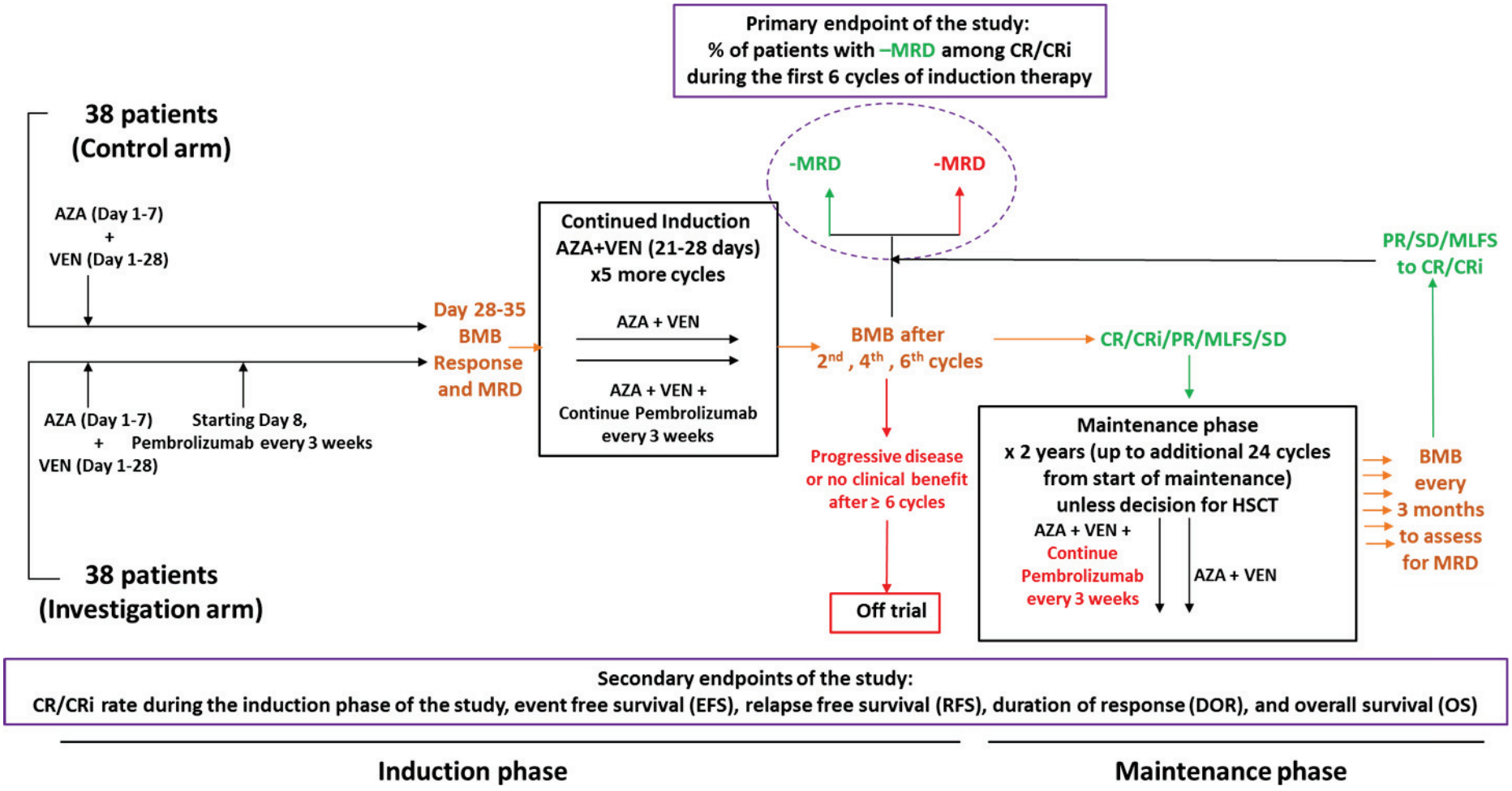
NCI-Supplied Agent: MK-3475 (pembrolizumab) (NSC # 776864)
Commercial Agents: Azacitidine (NSC # 102816), Venetoclax (NSC # 766270)

IND #:
IND Sponsor: DCTD, NCI

Protocol Type / Version # / Version Date: Original / May 30, 2019
Revision 1 / December 3, 2019

Revision 2 / January 9, 2020
Revision 3 / January 31, 2020
Revision 4a / March 11, 2020
Revision 5 / April 7, 2020
Revision 6 / May 14, 2020Revision 7 / June 9, 2020
Revision 8 / September 5, 2020 - Disapproved
Revision 9 / October 24, 2020
Revision 10 / October 11, 2021
Revision 11 / January 10, 2022
Revision 12 / April 11, 2022
Revision 13 / October 25, 2022
Revision 14 / February 10, 2023
Revision 15 / October 11, 2024
Revision 16 / November 27, 2024

SCHEMA



a. Complete Step 0: Initial Registration at time of sample submission.
b. Complete Step 1: Treatment Registration at time of completion of eligibility.
c. Patient may come off trial at any point after Induction to proceed to allogeneic stem cell transplant as per investigator discretion.

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1. OBJECTIVES

1.1 Primary Objectives

1.1.1 Assess the percentage of patients with minimal residual disease (MRD) negative CR (MRD-CR) or complete remission with incomplete count recovery (MRD-CRi) with AZA + VEN with pembrolizumab during the first 6 cycles and compare to control arm.

1.2 Secondary Objectives

1.2.1 Assess the investigator-assessed rates of CR/CRi/partial remission (PR)/morphological leukemia free state (MLFS) as defined per the modified International Working Group (IWG) 2003 response criteria with AZA + VEN with pembrolizumab (Cheson *et al.*, 2003), as well as rates of MRD negative MLFS.

1.2.2 Rates of complete remission with partial count recovery (CRh) and Hematologic improvement (HI) to red blood cells and platelets

1.2.3 Assess time to MRD negativity and duration of MRD negative state, event free survival (EFS), relapse free survival (RFS), calculated as the time from initial treatment to either disease relapse or death, duration of response (DOR, defined as the time from first CR/CRi to the date of the first documented relapse or death, whichever occurs first) and overall survival (OS).

1.2.4 Assess the proportion of patients who develop severe toxicity. Severe toxicity is defined as the occurrence of any of the adverse events (AEs) (section 9.5.1) during the first 35 days from start of Pembrolizumab (MK-3475) therapy

1.3 Exploratory Studies

1.3.1 MRD assessment by duplex sequencing (DS) and comparing DS and multiparameter flow cytometry for MRD detection as an exploratory biomarker.

1.3.2 Assessment of immune-checkpoint expression and dynamic change of immune cell subsets in response to the combination of checkpoint-inhibition and backbone combination in AML

1.3.3 High-throughput sequencing of the TCR Vb CDR3 regions on flow cytometrically sorted t-cell subsets to assess the effect of immunotherapy on the diversity of the t-cell repertoire and assess for correlation to clinical outcomes.

1.3.4 Investigation of protein signatures and RNA signatures associated with response and efficacy using O-link cytokine panel and RNA-seq, respectively

1.3.5 Determination of mutational load by whole exome sequencing to assess for correlation with clinical outcomes, immune infiltrating profile, and T cell repertoire diversity and clonality.

1.3.6. Profiling of DNA methylation patterns before and after treatment to assess for correlation to response to treatment.

1.3.7 Correlate gut microbiome at baseline and changes in the microbiome with clinical response, both in standard chemotherapy and immunotherapy/chemotherapy therapy settings.

1.3.8 MRD assessment using duplex sequencing strategy for circulating cell-free tumor DNA and correlation with long-term outcomes

1.3.9 Exploring different thresholds of MRD negativity using flow cytometry aside from the 0.1% level that will be used for the primary endpoint purposes (e.g. 0.01%).

2. BACKGROUND

2.1 Study Disease(s)

2.1.1 Acute myeloid leukemia (AML)

2.1.1.1 AML remains a disease with a significant unmet clinic need

Acute myeloid leukemia (AML) is a disease of myeloid precursors characterized by accumulation of immature myeloid progenitors, cells which lack the ability to differentiate, in the bone marrow interfering with normal hematopoiesis and leading to decreased production of normal blood cells (Dohner *et al*., 2015). AML is a disease of older adults with the median age at diagnosis is 67 years and one-third of patients are older than 75 years at time of diagnosis (Podoltsev *et al*., 2016). With the increasing population longevity, the number of newly diagnosed patients is increasing. In 2015, 20,830 patients were diagnosed with AML in the US, and more than 10,000 died from the disease. In patients who are younger than 60 years, the CR rates are 60% to 70% but the cure rates are only 35% to 40% (Dohner *et al*., 2010). However, older patients and those with adverse karyotypes have CR rates of 35% to 50% and cure rates of 10% or less. AML, especially in older patients, is characterized by high rates of chemotherapy resistance and therefore novel therapeutic approaches are urgently needed. Despite an explosion in understanding of the biology of AML and recent approval of midostaurin and gemtuzumab, the general therapeutic strategy for non-acute promyelocytic leukemia (APL) fit patients with AML remains intensive induction and consolidation chemotherapy and has not substantially changed in the last 40 years (Yates *et al*., 1973; Dohner *et al*., 2010; Kadia *et al*., 2016).

AML in elderly adults (>65 years of age) represent major therapeutic challenges, including both a more aggressive disease biology as well as a population that is more susceptible to the toxic effects of chemotherapy. Survival outcomes of AML in older patients are historically dismal, with a 3-year survival of < 10% (Menzin *et al*., 2002; Alibhai *et al*., 2009). Elderly patients are often not candidates for conventional cytotoxicity induction due to associated comorbidities and poor performance status (Pettit and Odenike, 2008). The de facto standard of care lower-intensity regimens for patients unfit for induction chemotherapy consist predominately of hypomethylating agents (HMAs) such as 5-azacitidine (azacitidine) and decitabine or low-dose cytarabine. Both the HMAs are currently approved by the Food and Drug Administration (FDA) for myelodysplastic syndrome (MDS) and commonly are used off-label for front-line or refractory disease in patients with AML. HMAs are especially used for older patients with high risk cytogenetics (Ravandi *et al*., 2009), however, median overall survival (OS) estimates with HMA monotherapy are poor at <1 year (Al-Ali *et al.*, 2012; Dombret *et al*., 2015; Cashen *et al*., 2010; Kantarjian *et al*., 2012). Furthermore, HMA monotherapy is associated with low response rates (10-50%), require several months to achieve best response, and are not curative (Dombret *et al*., 2015; Cashen *et al*., 2010; Kantarjian *et al*., 2012; Al-Ali *et al*., 2012). To this end, efforts are underway at developing rationally selected HMA combination approaches that can yield rapid clinical responses, which are more deep and durable (DiNardo *et al*., 2019).

2.1.1.2 Importance of measurable (also known as minimal) residual disease (MRD) in AML

Adult AML patients who do not have morphologic evidence of less than 5% of blasts on marrow examination after blood count recovery from induction chemotherapy are deemed to have attained a morphologic CR (Creutzig and Kaspers, 2004). However, without further therapy, the disease will recur in all patients and post-remission consolidation therapy remains a necessity demonstrating that residual leukemic cells remain in the bone marrow even after CR (Cassileth *et al*., 1988). The type of post-remission therapy is currently based on cytogenetic and molecular risk profiles (Dohner *et al*., 2010). Given that this risk assessment remains imperfect, there have been growing evidence regarding the value of achieving deeper responses and their assessment beyond morphologic assessment with the use of more sensitive laboratory measures (Paietta, 2012). This state, referred to as MRD, can be detected by multicolor flow cytometry (MFC) as well as genetic testing (*e.g.* polymerase chain reaction (PCR) or next-generation sequencing (NGS)) in cases where acquired genetic mutations or translocations were present at diagnosis. Monitoring response with MRD has been proven to be very useful and is now considered a standard practice in acute lymphoblastic leukemia (ALL), APL and chronic myeloid leukemia (CML), although its optimal use for therapeutic decisions in AML remains unknown (Santamaria *et al*., 2007; Hughes *et al*., 2010; Borowitz *et al*., 2015). Nevertheless, monitoring of MRD in AML with MFC is gaining popularity as there is clear evidence it refines risk assessment, and allows for long term monitoring for detection of early relapse (Hourigan *et al*., 2017). In a study by the AML Cooperative Group, achievement of negative MRD by MFC during aplasia after induction was highly predictive for 5-year outcomes (Kohnke *et al*., 2015). In two large prospective studies, the AML16 trial in the United Kingdom, and the HOVON SAKK AML 42 trial, undetectable MRD by MFC after 1 or 2 cycles of induction was significantly predictive for relapse risk and OS independent of other risk factors (Freeman *et al*., 2013; Terwijn *et al*., 2013). Although rates of MRD-negative CR achieved with induction therapy varies based on patient population studies and MRD detection assay used, in the two later clinical trials the percentage

of AML patients with MRD-negative CR was only around 50%. Though some patients who achieve MRD-negative CR still relapse, and some patients who have detectable MRD after induction therapy can subsequently become MRD-negative and achieve long-term survival, MRD negativity after induction chemotherapy is associated with better outcomes. The optimal cutoff to define MRD, the technology used for detection, the optimal timing for assessment (after induction or consolidation), and the incorporation in clinical decision making all remain undefined and are being studied in prospective clinical trials.

2.1.2 Immune Checkpoints and Immune Checkpoint Inhibition in AML

2.1.2.1 Role of the immune system activation in AML

The lower relapse rate associated with allogeneic hematopoietic stem cell transplantation (HSCT) and the ability of donor lymphocyte infusion (DLI) to rescue some patients who have relapsed following HSCT both demonstrate the role the immune system plays as the most potent anti-leukemia therapy in AML (Bleakley and Riddell, 2004; Kolb, 2008). Allo-reactive T-cells confer a strong graft-versus-leukemia (GVL) effect, however, HSCT continues to be associated with poor long-term survival with 3-year OS of only 41% and DFS of 37% (Tauro *et al*., 2005). Furthermore, HSCT is associated with significant morbidity and mortality and therefore many AML patients are not eligible for the procedure. Additionally, early results from investigational vaccine approaches in AML targeting tumor-associated peptide antigens such as WT-1 and PR1 further support the potential therapeutic benefit of recruiting the endogenous immune system (Chaise *et al*., 2008; Rezvani *et al*., 2008).

2.1.2.2 The role of the PD-1/PD-L1 pathway in AML

Programmed death receptor-1 (PD-1) is a molecule expressed on activated T-cells and delivers downstream signaling that causes inhibition of T-cell proliferation, cytokine release, and cytotoxicity (Okazaki *et al*., 2013). Recent data showed that multiple types of tumor are capable of evading the immune system by expressing PD-1 ligand (PD-L1), which engages the PD-1 receptor on the surface of cytotoxic T-cell leading to suppression of their activity (Pardoll, 2012). Immune checkpoint blockade with anti-PD-1 and anti-PD-L1 antibodies (and anti-cytotoxic T lymphocyte-associated protein 4 (CTLA-4) antibodies) has emerged as a novel promising approach to reverse this phenomenon (Postow *et al*., 2015). Immune checkpoint inhibition resulted in a significant and durable clinical activity in some patients with advanced solid malignancies including metastatic melanoma, non-small-cell lung cancer (NSCLC) and bladder cancer (Robert *et al*., 2014; Borghaei *et al*., 2015; Sharma *et al*., 2016) as well as in patients with Hodgkin's lymphoma (Ansell *et al*., 2015). Several lines of evidence suggest that PD-1/PD-L1 pathway is also of vital importance in AML.

2.1.2.2.1 Preclinical data: Immune checkpoint expression and inhibition in murine AML models

The importance of the PD-1/PD-L1 pathway in immune evasion of AML has been demonstrated in several murine AML models whereby PD-L1 and PD-1 were upregulated in murine leukemia cells and PD-1 blockade suppresses *in vivo* leukemia cell proliferation and improved survival in

AML bearing mice. (Zhang *et al*., 2009; Zhou *et al*., 2010). First, Zhang *et al* examined the expression of PD-L1 in the murine AML model C1498 (Zhang *et al*., 2009). The C1498 cell line was originally derived from a C57BL/6 mouse and most closely resembles AML. When murine C1498 AML cells were injected into syngenic C57BL/6 recipient mice intravenously (IV), leukemia cells grew progressively and evaded immune destruction. Low levels of PD-L1 expression were found on C1498 cells grown *in vitro*. However, PD-L1 expression was up-regulated on C1498 cells after a 48-hour incubation with interferon-gamma (IFN-γ) or when grown *in vivo* suggesting that the cytokine milieu within the leukemia "microenvironment" was capable of stimulating PD-L1 up-regulation. PD-1 knock out mice (PD-$1^{-/-}$) challenged with C1498 AML cells resulted in augmented antitumor T-cell responses measured by IFN-γ ELISpot assays performed from splenocytes isolated from wild-type and PD-$1^{-/-}$ mice 12 days after challenge with C1498 cells. This improved immune response resulted in a decreased AML burden in the blood and other organs of PD-$1^{-/-}$ mice and significantly longer survival compared to wild-type mice. Importantly, similar results were obtained with a PD-L1 blocking antibody, which decreased tumor burden, augmented an anti-leukemia immune response and prolonged survival of mice injected with C1498 AML cells. Second, Zhou *et al* used the same C1498 murine AML model focusing on the role of PD-1/PD-L1 interaction in regulatory T-cell (T_{reg})-mediated immune suppression of adoptively transferred CTLs (Zhou *et al*., 2010). They showed that PD-1 is upregulated on endogenous $CD8^+$ T-cells in the liver of AML-bearing mice and, consistent with Zhang's study, that the percentage of IFN-γ–producing cells is significantly decreased in the PD-1-expressing $CD8^+$ T-cell fraction compared with PD-1− fraction. AML progression caused significant accumulation of $CD4^+25^+FoxP3^+$ T_{regs} in the liver of wild-type (WT) mice, whereas PD-$1^{-/-}$ mice had a constitutively elevated level of T_{regs}, and this was not altered by the presence of tumor. T_{regs} from PD-$1^{-/-}$ mice were unable to suppress $CD8^+$ T-cell proliferation or IFN-γ secretion. Similarly, selective PD-1/PD-L1 blockade completely abrogated the ability of WT T_{regs} to suppress CD8+ T-cell proliferation IFN-γ production. These data indicated that both enhanced CD8+ T-cell response as a result of diminished suppressive function of PD-$1^{-/-}$ T_{regs} coupled with augmented PD-$1^{-/-}$ $CD8^+$ T-cells contribute to the resistance of tumor growth in PD-$1^{-/-}$ mice. Furthermore, they showed that anti–PD-L1 monoclonal antibody (mAb) administration enhances the efficacy of adoptive CTL therapy: while CTL therapy alone was ineffective, and anti–PD-L1 mAb treatment alone modestly prolonged the survival of but did not rescue AML-bearing mice, combined CTL and anti–PD-L1 mAb therapy in mice with advanced AML had an additive effect over either therapy alone and importantly resulted in 20% long-term survival.

2.1.2.2.2 Immune checkpoint expression in human AML samples

The expression of immune checkpoint seems to be only modestly elevated on AML cells at baseline but increases significantly, once AML cells are exposed to IFN-γ or chemotherapy and epigenetic therapy, as well as at the time of relapse either after chemotherapy or after bone marrow transplant (Kronig *et al*., 2014; Sehgal *et al*., 2015). Kronig *et al* demonstrated, in a cohort of 154 patients with AML, no significant increase in surface PD-L1 expression on leukemia cells at initial diagnosis compared to healthy controls (Kronig *et al*., 2014). However, stimulation with IFN-γ significantly increased PD-L1 expression on AML blasts but not in normal controls. Highest upregulation of PD-L1 upon IFN-γ as measured was observed in patients with CR and with relapse whereas only slight upregulation upon IFN-γ was found in

newly diagnosed patients. Yang *et al* showed that immune checkpoint expression was only modestly elevated on AML CD34+ cells at the time of diagnosis, however, was comparably higher when peripheral blood mononuclear cells (PBMCs) increased significantly once patients were treated with hypomethylating agents (Yang *et al*., 2014). Looking at the mRNA levels in $CD34^+$ cells from 32 patients with AML, they found that 15% had aberrant up-regulation (≥2-fold) in PD-L1, PD-1, and CTLA4, respectively. However, expression of PD-L1, PD-L2, PD-1, and CTLA4 in PBMCs from AML patients was significantly higher. Upregulation (≥2-fold) of PD-L1, PD-L2, PD-1, and CTLA4 was observed in 32%, 12.9%, 39%, and 3% of AML samples, respectively. Furthermore, treatment of leukemia cells with decitabine resulted in a dose-dependent upregulation of the above genes and patients resistant to therapy had relatively higher increments in gene expression compared with patients who achieved response. Exposure to decitabine resulted in partial demethylation of PD-1 in leukemia cell lines and human samples. Another small study identified PD-L1 to be preferentially expressed in AML-M5 and at higher surface levels during relapse compared to that at first diagnosis in patients (Chen *et al*., 2008). Norde *et al* found that in patients who relapsed late after allogeneic transplant, despite the presence of circulating alloreactive T-cells to hematopoietic cell-restricted minor histocompatibility antigens, PD-L1 was highly expressed on the leukemic cells at baseline or upon stimulation with IFN-γ (Norde *et al*., 2011). Furthermore, stimulation of allogeneic $CD3^+$ T-cells with the PD-L1-expressing AML cells led to significantly enhanced T-cell proliferation and cytokine production when performed in presence of anti-PD-1 antibody compared to isotype controls.

Based on these studies, one can speculate that PD-L1 expression is unlikely to be an oncogene-driven tumor immune escape mechanism but rather the result of an initially effective immune response against AML, which is later hampered by IFN-induced upregulation of PD-L1 interacting with PD-1-expressing immune cells (Kronig *et al*., 2014). As a form of adaptive resistance to therapy, AML cells co-opt the natural physiology of the PD-1/PD-L1 pathway for tissue protection in the face of inflammation, to protect itself from an anti-leukemia response.

2.1.2.2.3 Early clinical trials of checkpoint inhibition in AML:

A phase 1 trial using CT-011, a humanized antibody blocking with PD-1, in patients with advanced hematologic malignancies (AML, multiple myeloma (MM), Hodgkin lymphoma, and NHL) showed only limited activity (Berger *et al*., 2008). In a phase 2 trial, nivolumab has been combined with cytarabine and idarubicin for the treatment of 32 AML patients in the frontline setting (Ravandi *et al*., 2017). Treatment included 1 or 2 induction cycles of cytarabine 1.5 g/m^2 over 24 hours (Days 1-4) and idarubicin 12 mg/m^2 (Days 1-3). Nivolumab 3 mg/kg was started on Day 24 ± 2 days and was continued every 2 weeks for up to a year. Of the 32 patients, 23 patients (72%) achieved CR/CRi (19 CR, 4 CRi) and 9 patients went on to receive an allogeneic stem cell transplant (alloSCT). Among the patients proceeding to alloSCT the risk of graft versus host disease (GVHD) was not significantly increased. At baseline, bone marrow of non-responders had significantly higher percentage of $CD4^+$ T-effector cells co-expressing the inhibitory markers PD-1 and TIM3 ($p<0.05$) and a trend towards a higher percentage of $CD4^+$ T-effector cells co-expressing PD-1 and LAG3 compared to responders.

In the relapsed-refractory setting, a phase 2 trial examined the combination of high dose cytarabine followed by Pembrolizumab (MK-3475) (Zeidner *et al*., 2017). Thirteen patients with relapsed-refractory AML were to receive age-adjusted high dose cytarabine (HiDAC) (<60 years: 2 g/m^2 IV, every 12 hours [Q12H], Days 1-5; ≥60 years: 1.5 g/m^2 IV, Q12H, Days 1-5). This was to be followed by Pembrolizumab (MK-3475) 200 mg IV on Day 14 and maintenance phase Pembrolizumab (MK-3475) 200 mg IV every 3 weeks (Q3W) for up to 2 years until relapse/progression in case of a response. For the 10 evaluable patients at the time of abstract submission, overall response rate was 50% (CR/CRi: 4/10= 40%; partial response [PR]: 1/10=10%) and 2 patients proceeded to alloSCT. Post-alloSCT, both patients developed steroid-responsive acute GVHD of skin, one patient developed a transient increase in hepatic enzymes that was responsive to steroids, and one patient developed moderate chronic GVHD. Furthermore, a combination of the PD-1 inhibitor with the hypomethylating agent azacitidine (AZA) was shown to lead to improved response rates in the relapsed-refractory setting (Daver *et al*., 2016). In this study, patients with AML who had failed prior therapy, received AZA 75 mg/m^2 on Days 1-7 with nivolumab 3 mg/kg on Days 1 and 14. Median OS was 9.3 months, which compared favorably to historical survival with AZA-based salvage protocols in a similar patient population. Patients, who achieved a response, had baseline higher levels of total CD3, $CD8^+$ T-cells and a lower level of $CD4^+FoxP3^+PD\text{-}1^+T_{reg}$ cell infiltrate in the bone marrow. The ratio of $PD\text{-}1^+CD8^+$ T-effector cells to $PD\text{-}1^+CD4^+FoxP3^+Tr_{eg}$ cells was significantly higher in responders *vs*. non-responders. In summary, early results of phase 2 studies demonstrate the feasibility (with encouraging response rates) and safety of adding immune checkpoint blockade to chemotherapy or hypomethylating agents both in the frontline as well as the relapsed-refractory setting.

2.1.3 Hypomethylating agents modulate the immune system

The hypomethylating agents (HMAs), azacitidine and decitabine are valuable therapeutic options in older patients with AML deemed unfit for induction chemotherapy. Both of these agents, which initially were developed as antimetabolites, were later shown to target leukemogenic epigenetic changes (Fenaux *et al*., 2010). Mechanisms underlying response to HMAs include direct cytotoxic effects, terminal differentiation, and apoptosis (Juttermann *et al*., 1994). Mechanistic studies suggest that higher doses of therapy are primarily cytotoxic while hypomethylation activity is more prominent at lower levels of exposure (Christman, 2002). Apart from their cytotoxic and hypomethylating effects, these agents actively modulate the immune system, exerting a dual effect on anti-tumor immunity. In this context, an important mechanism of tumor immune response evasion by cancer cells lies in their ability to alter the expression of tumor-expressing antigens, resulting in deficient antigen presentation (Heninger *et al.*, 2015). HMAs can have a favorable effect on anti-tumor immune response by several mechanisms including upregulating a range of IFN-gamma pathway viral defense and immunomodulatory pathway-related genes such as cancer testis antigens, increasing class I HLA expression on tumor cells, and upregulating co-stimulatory molecules (CD28, CD40L) (Li *et al*., 2014; Srivastava *et al*., 2016; Coral *et al*., 1999; Coral *et al*., 2002). In addition to the favorable effects on anti-tumor immunity, HMA treatment can also dampen immune response by upregulating the expression of inhibitory check point receptor PD-1 on T cells and inhibitory ligands PD-L1 and PD-L2 on tumor cells. The increased expression of PD-1 on T cells is brought about by azacitidine induced hypomethylation of the PD-1 promotor region

(Youngblood *et al*, 2011). The increased PD-L1 expression on tumor specific T cells, if left unchecked, promotes their exhaustion.

In a study by Ørskov *et al*, treatment with azacitidine was accompanied by DNA demethylation in 44% of patients with AML/MDS. Hypo/demethylation of the PD-1 promoter status was correlated with significantly worse response rates and a trend toward a shorter OS (Ørskov *et al*, 2015). In another study by Yang *et al*, azacitidine upregulated PD-1 and PDL1 mRNA expression on the PB mononuclear cells in approximately 50% of patients with AML or MDS. PD-1 and its ligands, PD-L1 and PD-L2, and to a lesser extent CTLA-4, were also aberrantly upregulated (≥2-fold) on BM CD34 + cells by mRNA expression, in approximately 30–40% of patients with AML or MDS (Yang *et al*, 2014). Patients resistant to azacitidine-based therapy had relative higher increments in gene expression on the PB mononuclear cells and BM CD34 + cells as compared with patients who achieved response, suggesting this could be a mechanism of resistance (Yang *et al*, 2014). Azacitidine also enhances anti-tumoral activity of the anti-CTLA-4 antibody by inducing hypomethylation of human endogenous retroviral elements eventually leading to upregulation of immune effector pathway signaling genes, thereby leading to sensitization to anti-CTLA4 antibody therapy (Chiappinelli *et al*., 2017; Roulius *et al*., 2015). Apart from their effects on tumor antigen and immune check point receptor-ligand expression, HMAs can also impact T-cell numbers and phenotypes such as increasing peripheral blood T-reg cells, decreasing Th1 and Th2 cells, and shifting CD8 lymphocytes towards CD8+/IFN-gamma+ T subtype (which expresses a higher level of PD-1) (Catakovic *et al*., 2017; Costantini *et al*., 2013).

Given that the immune check point molecular upregulation represents an important mechanism of HMA resistance, a number of clinical trials combining HMAs with PD-1/PD-L1- based therapies have recently started enrollment for AML and MDS, including azacitidine with the anti-PD-1 antibody nivolumab (NCT02397720). This phase II study enrolled 73 patients with R/R AML; the median age was 70-years (range,22-90) and the median number of prior therapies was 2 (range,1-7) (Daver *et al*., 2018b). The ORR was 58% and 22%, in HMA-naive (n=25) and HMA pre-treated (n=45) patients, respectively. Notably, grade 3-4 immune-related adverse events (irAE) occurred in eight (11%) patients. The irAE profile differed slightly from that seen in solid tumors with the more common irAEs being pneumonitis, nephritis, colitis, and dermatitis compared to endocrinopathies, skin rash, and transaminitis described more frequently in solid tumors. Pre-therapy bone marrow and peripheral blood CD3 and CD8 were significantly predictive for response on flow-cytometry (Daver *et al*., 2018b). CTLA-4 was significantly up-regulated on CD4+ T effector cells in non-responders after 2, 4, and 6 doses of nivolumab suggesting that combination blockade of both these major co-inhibitory pathways may improve response rates and durability of responses.

The combination of azacitidine with nivolumab was also evaluated in frontline MDS setting and demonstrated to be effective, with a response rate of 80% (NCT02530463) (Garcia-Manero *et al.*, 2016). This phase 2 study also evaluated nivolumab alone and ipilimumab alone in patients with MDS who had failed prior therapy with a HMA. Interestingly, while ipilimumab demonstrated response in 33% of patients with high risk relapsed/refractory MDS, nivolumab showed no single agent activity suggesting that there may be a differential efficacy profile for PD-1 and CTLA-4 inhibition in myeloid diseases. Overall, clinical trial data from HMA-ICPi

combination approaches have shown encouraging response rates and durable responses without resorting to stem cell transplant.

2.2 CTEP IND Agent

Pembrolizumab (MK-3475) has high affinity and potent receptor-blocking activity for the programmed cell death 1 (PD-1) receptor, based on preclinical *in vitro* data (Investigator's Brochure, 2018). Pembrolizumab (MK-3475) has an acceptable preclinical safety profile and is being advanced for clinical development as an IV immunotherapy for advanced malignancies.

The importance of intact immune surveillance function in controlling outgrowth of neoplastic transformations has been known for decades (Disis, 2010). Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes in cancer tissue and favorable prognosis in various malignancies. In particular, the presence of $CD8^+$ T-cells and the ratio of $CD8^+$ effector T-cells/$FoxP3^+T_{regs}$ correlates with improved prognosis and long-term survival in solid malignancies, such as ovarian, colorectal, and pancreatic cancer; hepatocellular carcinoma; malignant melanoma; and renal cell carcinoma. Tumor-infiltrating lymphocytes can be expanded *ex vivo* and re-infused, inducing durable objective tumor responses in cancers such as melanoma (Dudley *et al.*, 2005; Hunder *et al.*, 2008).

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene *Pdcd1*) is an Ig superfamily member related to cluster of differentiation 28 (CD28) and CTLA-4 that has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2) (Greenwald *et al.*, 2005; Okazaki *et al.*, 2001).

The structure of murine PD-1 has been resolved (Zhang *et al.*, 2004). PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable–type (IgV type) domain responsible for ligand binding and a cytoplasmic tail responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif, and an immunoreceptor tyrosine-based switch motif. Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases, SHP-1 and SHP-2, to the immunoreceptor tyrosine-based switch motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3 zeta (CD3ζ), protein kinase C-theta (PKCθ), and zeta-chain-associated protein kinase (ZAP70), which are involved in the CD3 T-cell signaling cascade (Chemnitz *et al.*, 2004; Sheppard *et al.*, 2004; and Riley, 2009). The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from, that of CTLA-4, because both molecules regulate an overlapping set of signaling proteins (Parry *et al.*, 2005; Francisco, 2010). As a consequence, the PD-1/PD-L1 pathway is an attractive target for therapeutic intervention in AML.

2.2.1.1 Pembrolizumab (MK-3475) Background and Clinical Trials

Pembrolizumab (Keytruda®), a humanized monoclonal antibody against the PD-1 protein, has been developed by Merck & Co. for the treatment of cancer. Pembrolizumab (MK-3475) is approved for treatment of melanoma in several countries; in the United States (US) and European Union (EU) it is approved for the treatment of advanced (unresectable or metastatic) melanoma in adults. Pembrolizumab (MK-3475) has also been approved for treatment of NSCLC in several countries; in the US it is indicated for the treatment of patients with metastatic NSCLC whose tumors express PD-L1 as determined by a Food and Drug Administration (FDA)-approved test and who have disease progression on or after platinum-containing chemotherapy. Patients with NSCLC and epidermal growth factor receptor (EGFR) or anaplastic lymphoma kinase (ALK) genomic tumor aberrations should also have disease progression on FDA-approved therapy for these aberrations prior to receiving Pembrolizumab (MK-3475). Pembrolizumab (MK-3475) is approved in the US for the treatment of patients with recurrent or metastatic head and neck squamous cell carcinoma (HNSCC) with disease progression on or after platinum-containing chemotherapy.

Pembrolizumab (MK-3475) has demonstrated initial clinical efficacy in single-arm monotherapy trials in patients with NSCLC, HNSCC, urothelial cancer, gastric cancer, triple negative breast cancer, and Hodgkin's Lymphoma as determined by response rate. Ongoing clinical trials are being conducted in these tumor types as well as a number of other advanced solid tumor indications and hematologic malignancies. For study details please refer to the Investigator's Brochure (2018).

2.3 Other Agent(s)

2.3.1 Venetoclax

B-cell lymphoma 2 (BCL-2) is a mitochondrial pathway anti-apoptosis protein that plays a key role in survival and persistence of AML blasts (Vo *et al.*, 2012; Konopleva *et al.*, 2006). BCL-2 overexpression has been implicated in chemotherapy resistance and inferior overall survival in AML (Konopleva *et al.*, 2006). Venetoclax (ABT-199/GDC-0199) is a potent and highly selective small molecule BCL-2 inhibitor that has shown preclinical and clinical activity across a range of hematologic malignancies, including AML. Targeted BCL-2 inhibition with venetoclax has shown to induce cell death in AML cell lines and leukemic blasts and stem cells while sparing normal hematopoietic stem cells. (Vo *et al.*, 2012; Konopleva *et al.*, 2006; Lagadinou *et al.*, 2013). In the relapsed-refractory (R/R) AML setting, a phase 2 study examined the single agent activity of venetoclax. The CR rate was 19% with another 19% having some form of myeloblast reduction. The drug was well tolerated with AEs consistent with expectations in the R/R AML population. Following its modest clinical activity and acceptable safety profile as monotherapy, venetoclax was investigated in phase 1 studies in combination with HMAs (decitabine or azacitidine) (NCT02203773) (Konopleva *et al.*, 2016). This drug combination strategy is based on *ex vivo* pre-clinical evidence that combining venetoclax with azacitidine was potently synergistic in inducing AML cell death (Bogenberger *et al.*, 2015). Studies suggest that azacitidine downregulates anti-apoptotic Mcl-1 protein concentrations, which is a known mediator of resistance to venetoclax (Tsao *et al.*, 2012). Furthermore, the combination was shown to induce apoptosis in a p53 independent manner which has potential therapeutic implications in *TP53* AML (Tsao *et al.*, 2012).

A combination of venetoclax with hypomethylating agents in treatment naïve elderly (65 years or above) AML was shown to lead to high response rates, which were comparable to standard induction therapy, with acceptable toxicity (DiNardo *et al.*, 2018a). In the final report of this phase 1b AML study 145 patients were enrolled and received oral venetoclax in combination with either decitabine (20 mg/m^2, Days 1-5) or azacitidine (75 mg/m^2, Days 1-7) (DiNardo *et al.*, 2019). In the expansion phase, venetoclax 400 mg or 800 mg with either HMA was given. With a median duration of follow up of 15.1 months (range, 9.8-31.7), 67% and 83% of the total study population had achieved a CR/CRi (97 of 145) and overall leukemia response rate (CR+CRi+partial remission+median leukemia free state), respectively. Response rates were similar between the venetoclax 400 mg + azacitidine and venetoclax 400 mg + decitabine cohorts. Notably, responses were noted even in high risk patient groups, such as poor-risk cytogenetics, ≥75 years, and secondary AML. Median times to best response (CR/CRi), duration of CR/CRi, and OS in the overall population were 2.1, 11.3, and 17.5 months, respectively; mOS has not been reached for the 400 mg venetoclax cohort. The CR/CRi rate, median duration of CR/CRi, and mOS for venetoclax 400 mg + azacitidine cohort were 76%, not reached (NR) (95% CI, 5.6-NR), and NR (95% CI, 9.0-NR), respectively. Data obtained from multiparameter flow cytometry ($<10^{-3}$) demonstrated MRD negativity, at least once during study treatment, in 29% of patients with CR/CRi. In summary, the CR+CRi rate of 67% and the mOS of 17.5 months achieved with the HMA-venetoclax combination is notable compared with CR+CRi rate and mOS of 10-50% and <12 months, after HMA monotherapy (Dombret *et al*., 2015; Cashen *et al*., 2010; Kantarjian *et al*., 2012; Al-Ali *et al*., 2012). Based on these data, the FDA granted approval in November 2018 of the combination of HMAs with venetoclax in management of untreated older patients who are unfit for intensive chemotherapy.

2.3.1.1 Venetoclax's effects on the immune system

The BCL-2 family of proteins play a crucial role in the maintenance and survival of immune cells, as well as in regulating anti-tumor responses of the immune system (Bougras *et al.*, 2004; Hawkins and Vaux, 1997). Given the critical role of BCL-2 in immune system regulation, venetoclax may affect the anti-tumor activity of immune checkpoint inhibitors. Two recent reports investigated the effects of venetoclax mediated BCL-2 inhibition on the viability and function of immune-cell subsets (Mathew *et al.*, 2018; Lasater *et al.*, 2018). Data from these studies suggest that venetoclax treatment results in loss of B-cell and naïve T-cell subsets but not memory CD8+ and CD4+ T effector cells. The lack of impairment in survival or activation of resting CD8+ T-cell memory subsets is possibly due to increased levels of BCL-X_L and MCL-1 (Lasater *et al.*, 2018). In mixed lymphocyte reaction and cytomegalovirus recall assays, venetoclax did not affect the viability, the induction or frequency of memory T cells (Mathew *et al.*, 2018). Furthermore, venetoclax did not antagonize the therapeutic effect of anti-PD-1 in *in vivo* syngeneic murine models (Mathew *et al.*, 2018; Lasater *et al.*, 2018). On the contrary, venetoclax-exposed memory T-cells were able to rapidly acquire effector and cytotoxic function to eliminate cancer cells, thus supporting the rationale for combinability of venetoclax with immune check point inhibitors (Mathew *et al.*, 2018). The enhanced efficacy of the venetoclax-anti-PD-1 combination appears to be immune-mediated in nature (Mathew *et al.*, 2018). To best of our knowledge, the venetoclax-ICPi combination strategy is yet to be explored in clinical trials.

2.3.2 Azacitidine

Azacitidine (Vidaza®, NSC 102816) is a pyrimidine nucleoside analogue of cytidine that is incorporated into both RNA and DNA, leading to loss of DNA methylation and cytotoxicity (Stresemann and Lyko, 2008). Azacitidine is metabolized to 5-aza-2'-deoxycytidine triphosphate after cellular uptake, incorporated into DNA in place of cytosine, and forms covalent bonds to DNA methyltransferases (DNMT), leading to loss of DNA methylation by impairing DNMT function and triggering of DNA damage signaling and DNMT degradation. For more detailed information, please consult the Vidaza® package insert (2016).

2.3.2.1 Azacitidine activity in treatment-naïve, relapsed or refractory AML, and AML with MRD

Epigenetic changes, including DNA methylation, play an important role in myeloid leukemogenesis, and the DNA hypomethylating agent azacitidine targets aberrant DNA methylation in AML (Döhner *et al.*, 2015). A subset analysis of the AZA-001 study, which compared azacitidine to conventional care for patients with MDS, showed a CR rate of 17% for azacitidine-treated patients (Fenaux *et al.*, 2009). The AZA-001-AML study compared azacitidine to conventional care for older patients with AML with >30% blasts and showed a CR/complete response with incomplete hematologic recovery (CRi) rate of 27.8% and a trend towards improved OS (Dombret *et al.*, 2015). In both studies, azacitidine was well tolerated with primarily hematologic toxicity. Several studies have also demonstrated clinical activity of azacitidine in R/R AML (Craddock *et al.*, 2016; Itzykson *et al.*, 2015; Ivanoff *et al.*, 2013; Roboz *et* al., 2014; Tawfik *et al.*, 2014). The results are summarized in Table 1. In a phase 3 study comparing elacytarabine to investigator choice, the azacitidine arm showed a median OS of approximately 6-7 months and 12-month survival of 25%. CR/CRi rate was not reported. Other studies evaluating the activity of azacitidine in R/R AML showed CR/CRi rates ranging from 4-21%, median OS 8.2-9 months, and 12-month survival of 25-30%. These studies included a single center experience, a trial in patients after prior intense chemotherapy, a trial in older patients, and a trial after allogeneic HCT.

Table 1: Azacitidine efficacy in R/R AML.

Study	**CR/CRi**	**Median OS**	**1-year Survival**
Phase 3	NR	7 months	25%
Single-Center	4%	8.2 months	25%
After prior intense chemotherapy	21%	9 months	30%
Elderly patients	17%	8.4 months	25%
After allogeneic HCT	15%	NR	12% (2-year)
NR = Not reported; CR/CRi = complete response plus complete response with incomplete count recovery; OS = overall survival; HCT = hematopoietic cell transplant.			

Measurable/minimal residual disease (MRD) refers to low levels of disease that can be detected in some patients without frank hematologic relapse (<5% blasts). Methods used to measure MRD include multiparameter flow cytometry (MFC), real-time quantitative polymerase chain reaction (RT-qPCR) and targeted next-generation sequencing (NGS). Several studies have shown that detection of MRD after CR is highly prognostic for survival. For example, in one

study examining a large cohort of patients ages <60 years, presence of MRD after induction was associated with a 43% 5-year OS *versus* 63% in those without MRD (Freeman *et al.* 2017). Another study showed that MRD detected by both MFC and targeted NGS were independently prognostic and together had additive prognostic value (Jongen-Lavrencic *et al.* 2018). Importantly, several studies are congruent in showing that pre-transplant MRD predicts for survival similar to that of going to transplant with active AML. For example, in one study of 359 patients (median age at transplant = 50 years) in MRD negative CR, MRD positive CR, or with active AML before allogeneic transplant, the 3-year OS was 73% for MRD negative patients compared to 26% for MRD positive patients and 23% for patients with active AML (Araki *et al.*, 2016). Thus, improving outcomes for patients with detectable MRD has the potential to prevent or delay relapse and improve survival. To this end, the RELAZA2 trial showed that azacitidine can prevent or delay hematologic relapse in patients (median age 59, range 52-69) with AML in CR with very early relapse as evidenced by newly detectable MRD. Treatment with standard-dose azacitidine led to a 58% response rate after six cycles and prevented relapse in 51% of patients. The 12-month relapsed free survival (RFS) and OS was 46% and 75%, respectively (Platzbecker *et al.*, 2018). Overall, these findings support the further evaluation of azacitidine-based therapy as an approach to delay or prevent relapse and improve outcomes after transplant in patients with very early relapse (<5% blasts).

2.4 Rationale

2.4.1 Rationale for combining azacitidine + venetoclax combination therapy with immunotherapy (anti-PD-L1 antibody pembrolizumab) to stimulate immune attack against AML blasts

The recent trial data from the azacitidine + venetoclax (AZA + VEN) combination in front-line AML suggests a high clinical activity with complete response rates approaching 70% and durable responses, with a median duration of complete response of 11.3 months (DiNardo *et al.*, 2019). Furthermore, 30% of the complete responders were MRD negative. Combining AZA + VEN combination chemotherapy with an immune check point inhibitor (ICPi) could lead to synergy by combining the immunomodulatory abilities of checkpoint inhibition and the AZA + VEN. Chemotherapy with AZA + VEN as the backbone leads to immunogenic cell death of leukemic blasts resulting in antigen release and cross presentation of antigens by dendritic cells with a priming effect on CTL (Kroemer *et al*., 2013; Zitvogel *et al*; 2013). Interferon-gamma (INF-γ) leads to an activation of T cells but also an increased expression of PD-L1 on leukemic blasts (Blank *et al*., 2004; Chen *et al*., 2012). Subsequently, CTLs are not able to kill leukemic blasts because they are inactivated by an increased expression of PD-L1 on leukemic blasts resulting in therapy resistant tumor cells or relapse after completion of chemotherapy. Furthermore, induced expression of immune checkpoint pathway-related genes (PD-1, PD-L1, PD-L2) by HMAs leads to exhaustion of T cells and is a frequently described immune evasion mechanism of HMA resistance. The functionality of these 'exhausted' T cells may be restored by concomitant PD-L1/PD-L1 blockade. Furthermore, venetoclax-exposed CD8+ memory T-cells are able to rapidly acquire effector and cytotoxic function to eliminate cancer cells thus supporting the rationale for combinability of venetoclax with ICPis. Adding the anti PD-1 antibody pembrolizumab to AZA+ VEN chemotherapy may lead to more effective CTL mediated destruction of leukemic blasts. The goal with combining pembrolizumab to AZA+

VEN chemotherapy is to facilitate a more effective CTL mediated destruction of leukemic blasts resulting in improved rates of CR without MRD, which would hopefully increase duration of response and lower relapse rates.

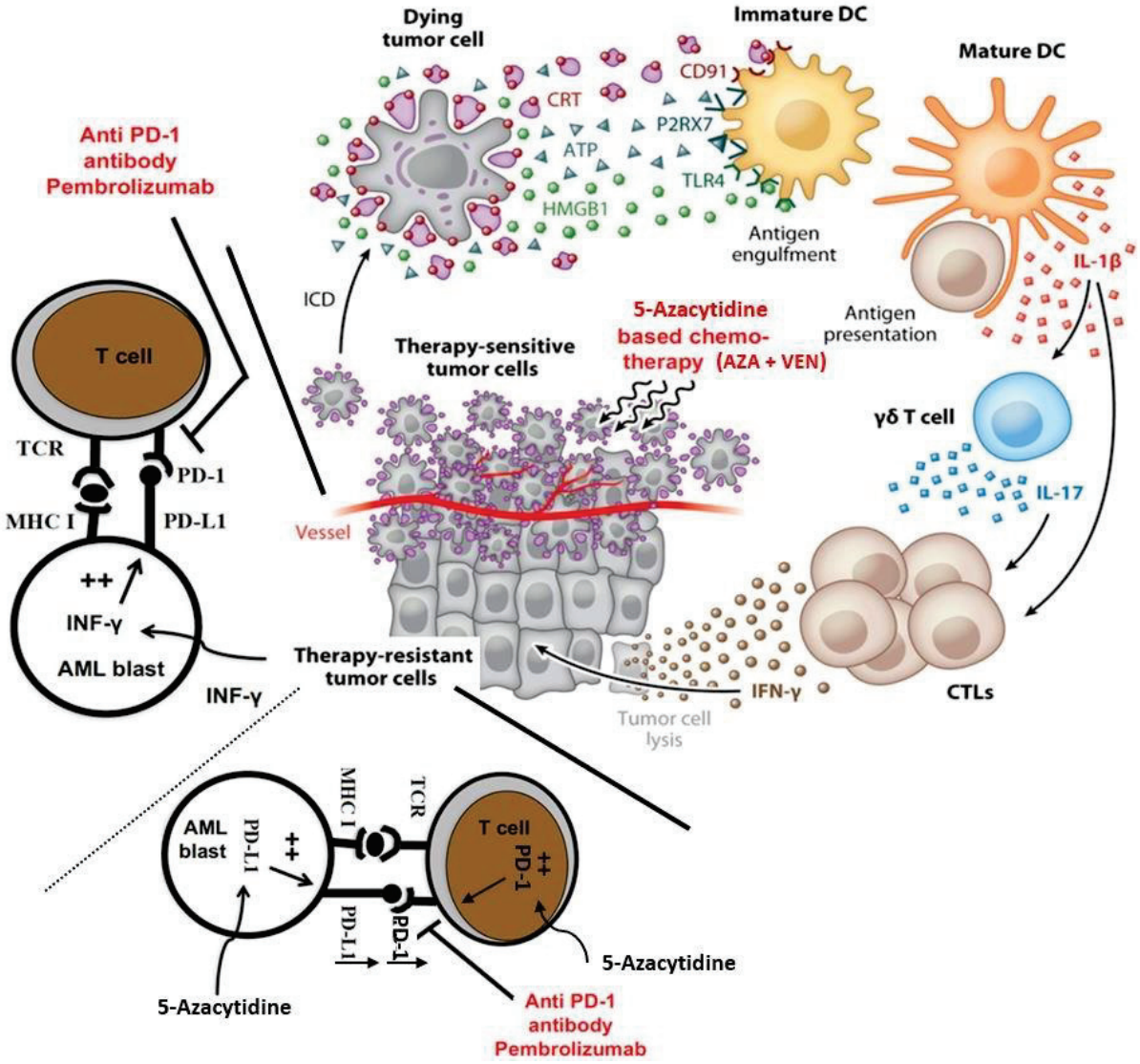


Figure 1: Potential synergistic effect of azacitidine + venetoclax combination and checkpoint inhibition with anti PD-1 antibody pembrolizumab in inducing an immune response against AML blasts. (Kroemer *et al*., 2013; Daver *et al*., 2018a)

2.4.2 Safety of the combination of epigenetic and therapy and chemotherapy with checkpoint inhibition

Combination of checkpoint inhibition with chemotherapy and targeted therapy has been shown to be safe in solid tumors (Morrissey *et al.*, 2016). For instance, in NSCLC nivolumab has been successfully combined with platinum doublet chemotherapy (Rizvi *et al.*, 2016). Checkpoint inhibitors have been combined with chemotherapy (high dose cytarabine followed by pembrolizumab) (Zeidner *et al.*, 2017) and epigenetic therapy (azacitidine and nivolumab)

(Daver *et al.*, 2017) in relapsed and refractory AML. Similarly, checkpoint inhibitors have been combined with chemotherapy (cytarabine, idarubicin, and nivolumab) (Ravandi *et al.*, 2017) and epigenetic therapy (azacitidine and nivolumab) (Daver *et al.*, 2017) for the treatment of AML patients in the frontline setting. The combination of checkpoint inhibitors with chemotherapy or epigenetic therapy was shown to be feasible and safe in the studies (Zeidner *et al.*, 2017; Daver *et al.*, 2017). Importantly, preliminary results from AML trials using anti-PD1 therapy in combination with intensive chemotherapy in the relapsed/refractory setting (Zeidner, 2017) and high doses of cytarabine/idarubicin in frontline setting (Ravandi *et al.*, 2017) and in combination with HMAs (Ørskov, et al; 2015) among the patients who proceeded to HSCT after checkpoint inhibitor therapy found NO signal for increased risk of graft-versus-host disease or transplant-related toxicity (Ravandi *et al.*, 2017a; Zeidner *et al.*, 2017; Daver *et al.*, 2017). Therefore, it is highly unlikely that there would be an increase in post-transplant toxicities using this combination among the very few patients who might proceed to HSCT.

2.5 Standard of care assessments:

2.5.1.1 *FLT3* mutations

FLT3 represents the most common mutated gene in AML, with over 90% of AML expressing a *FLT3*. Mutations in the *FLT3* can either be internal tandem duplications (*FLT3-ITD*) within the juxta-membrane domain or point mutations within the kinase domain (*FLT3*-TKD). *FLT3*-ITD mutations can be detected in 25-30% of AML cases and carry a worse prognosis (El Fakih *et al.,* 2018; Wang *et al.,* 2005). Patients with newly diagnosed *FLT3-ITD* mutated AML who are treated with conventional chemotherapy have poorer survival outcomes, with 3-year survival estimates <20%, than do patients without these mutations due to their increased risk of relapse and inferior disease-free survival (Thiede *et al.,* 2002). A number of other FLT3 inhibitors (such as quizartinib, gilteritinib, and midostaurin) are currently undergoing clinical development in phase 3 trials. In 2018, midostaurin received breakthrough therapy designation from the FDA for newly diagnosed *FLT3*-mutated AML, after demonstrating significantly improved OS in a randomized phase 2 study (RATIFY) of induction and consolidation chemotherapy combined with midostaurin or placebo (5-year OS: 51% *vs.* 43%; p=0·007) (Stone *et al.,* 2017). A FLT3 inhibitor (midostaurin) in combination with 7+3 is now the standard of care in the management of newly diagnosed *FLT3*-mutated AML for patients who undergo intensive chemotherapy. However, as the use of FLT3 inhibitors in older unfit patients who are not receiving intensive chemotherapy is not a standard of care or defined yet, those patients will be allowed on the trial. Given FLT3 assessment will not be required for eligibility or stratification, the results of FLT3 testing are not required to be available at time of screening or randomization as they patients will not be getting intensive chemotherapy.

2.5.2 Cytogenetics

The clinical and molecular heterogeneity of AML brings about varied response to induction chemotherapy, as well as drug resistance and disease relapse. The current risk-stratification systems (such as ELN-2017 and NCCN-2018) classify AML into favorable, intermediate, and adverse risk categories based on therapeutic sensitivity to standard chemotherapeutic approaches and likelihood of sustained remission after induction and consolidation. Core-binding factor

(CBF) AML is a favorable risk AML category characterized by t(8;21)(q22;q22) and inv(16)(p13;q22)/t(16;16)(p13;q22) abnormalities, and despite mutational and cytogenetic heterogeneity among t(8;21) and inv(16), these entities share similarities in prognosis, are grouped together, and managed similarly (Appelbaum *et al*., 2006). In patients in favorable risk AML (Othus *et al.,* 2014) treated with conventional chemo induction, clinical trial data have reported CR and long-term survival rates approaching 80-90% and 50-65%, respectively (Paschka *et al.,* 2018; Schiller, 2005). While categorization into intermediate-risk AML is primarily based on cytogenetics, the definition of this broad and heterogenous category continues to evolve, as reflected by the incorporation of molecular markers of prognostic importance such as *FLT3, NPM1,* and *CEBPA.* Unlike the case with the favorable and intermediate risk AML, adverse risk categories represent a particularly hard-to-treat disease population characterized by lower remission rates, high early mortality, and early relapse and death. Analysis and interpretation of outcomes between newer treatment approaches and traditional cytotoxic chemotherapy in AML must therefore be within the context of the underlying AML risk status. Therefore, we usecytogenetics as a stratification factor to account for heterogeneity in outcomes based on AML risk status. *NPM1, FLT3, CEBPA* and other genetic alternations are required for the risk group assignment in ELN and NCCN risk stratifications. Since the results of tests for these molecular markers are often not available at time of therapy initiation, we will use a risk stratification system that relies only on cytogenetic abnormalities for sake of stratification at randomization and not one that incorporates molecular markers. Patients with core binding factor (CBF) AML, rare among older patients, will be excluded. Patients with no-CBF AML will be stratified into two groups based on cytogenetic results available at time of randomization: Adverse cytogenetics vs. intermediate/unknown risk karyotype based on the NCCN/ELN karyotype risk stratification.
Some patients with AML require initiation of therapy quickly after diagnosis, and full metaphase karyotype results in some centers can take 2-3 weeks to result. To avoid this issue being an impediment to accrual to study or to cause unwanted delays in initiation of therapy in patients who need fast initiation of therapy, we will allow use of karyotype and/or FISH results (as well as FLT3 results) on samples from blood or marrow that were obtained up to 3 weeks before signing consent for purposes of eligibility and stratification. Adverse karyotype can be determined based on FISH results (e.g. loss of chromosome 7 or 5 or 3 or more abnormalities) based on the specific probes used in the FISH. If results of full metaphase karyotype are not available and the available FISH results do not suggest an adverse karyotype, and there is a need to initiate therapy before those full results are available, then the patient can be stratified into the unknown/intermediate group for randomization purposes. In any case, results from FISH or karyotype should show that CBF abnormalities are NOT present by at time of randomization as the presence of CBF abnormalities is an exclusion factor.

Refer to Section 9.3 for further details on stratification factors.

2.6 Correlative Studies Background

2.6.1 Multiplex Immunofluorescence (mIF)

Immunohistochemistry is one of the most acceptable tools to measure PD-1 and PD-L1 expression in tumor microenvironment (TME). Human BM core biopsy will be decalcified for immunohistochemistry (IHC). We will compare IHC/IF findings and its quantitative analyses by Multiplex Immunofluorescence (MiF) (will be describe below) with flow cytometric data in BM aspirate and blood. All these procedures will be performed with a collaboration with Dr. Ignacio I Wistuba at MDACC CIMAC. For multiplex IF image analysis platform, we will use the tyramide signal amplification (TSA) methodology through the Opal™ workflow and chemistry which allows simultaneous staining of multiple biomarkers within a single paraffin tissue section. The multispectral microscopy Vectra™ and Vectra-Polaris™ scanner systems (Akoya Biosciences, Waltham, MA, USA) including the image analysis software (InForm, Akoya Biosciences) allows the analysis of the multispectral images generated by this staining methodology. Multiplex ImmunoFluorescence (IF) staining will be performed on FFPE tumor slides using the following panels and antibodies:

1. Panel 1 (PD-L1/PD-1 and TIL Panel): PD-L1, CD68, PD-1, CD8, CD3, PanCytokeratin, DAPI.

2. Panel 2 (T cell Activation/ Regulatory Panel): FOXP3, Granzyme B, CD45RO, CD8, CD3, PanCytokeratin, DAPI.

2.6.2 Mass Cytometry (CyTOF)

We will possibly have limited number of cells in the BM aspirate. Therefore, we will analyze the immune profile combining a high-throughput technique that will allow us to study more than 20 markers in a single cell analysis using CyTOF. These techniques will provide information about the architecture of TME (*i.e*. BM) and the distribution of markers of interest detected with CyTOF. CyTOF-based single cell analysis is a new technology that combines flow cytometry with metal-conjugated antibodies detected by mass spectrometry permitting higher order multiplexing (up to 100 molecules) on a single cell analysis (Bendall *et al*., 2011). For this assay, the staining will be performed using MaxPar Human T-Cell Phenotyping Panel Kit (CD11a, CD4, CD8a, CD16, CD25, CD45, CCR7, CD69, CD45RO, CD44, CD27, CD45RA, CD3, CD57, HLA-DR, and CD127; along with CD28, CD117, and CD95), modified MaxPar human AML phenotyping panel (added CD10,CD13, CD16 on top of CD19, CD117, CD11b, CD64, CD7, CD123, CD45, CD33, CD15, CD34, CD3, CD44, CD38, HLA-DR, and CXCR4) for surface staining as per manufacturer's protocol. These markers may change as the technology evolves and develops. Additionally, after fixation and permeabilization, cells will be stained with anti-human Ki-67-151Eu (B56; BD Pharmingen Ab conjugated with lanthanide MaxPar Europium Chloride 151Eu using the MaxPar X8 Ab labeling kit) for 30 min at room temperature. Cells will be acquired on CyTOF 2 instrument (DVS; Fluidigm Sciences). All data will be analyzed and graphs generated using the DVS Cytobank software (Cytobank)

2.6.3 Olink cytokine panel

Inflammation is a key underlying factor for the pathophysiology of a wide range of diseases, including malignancies. The Olink inflammation biomarker panel provides a high-throughput,

multiplex immunoassay enabling analysis of 92 inflammation-related protein biomarkers across 96 samples simultaneously. The objective is to investigate protein signatures associated with response and efficacy. We will perform this procedure on the peripheral blood in collaboration with Dr. Sacha Gnajtic at Mount Sinai CIMAC.

2.6.4 Whole Exome Sequencing

The determination of the mutation load, a total number of nonsynonymous point mutations, by whole-exome sequencing (WES) was shown to be useful in predicting the treatment responses to cancer immunotherapy (Lyu, 2018). Tumor mutational burden (TMB), along with PD-L1 expression, is a useful biomarker for immune checkpoint blockade selection across certain cancer types and TMB determination correlated with WES (Chan, 2019). We will use massively parallel sequencing technology to sequence the genomic DNA of tumor cells (leukemic bone marrow) and normal cells (germline) obtained from patients with AML at baseline. Mutational load by WES will be correlated with clinic-pathological parameters such as response to treatment, survival and immune infiltrating profile, and T cell repertoire diversity and clonality.

2.6.5 RNA-seq

The MD Anderson CIMAC uses Agilent RNA isolation products for RNAseq. RNA will be prepared using suitable purification system depending on sample source (fresh or FFPE). RNA integrity of FFPE RNA will be assessed using either the Agilent 4200 TapeStation and High Sensitivity RNA ScreenTape or the Agilent 2100 Bioanalyzer and RNA 6000 Pico Chip. Either method will employ the region analysis method to determine the percentage of RNA in the sample that is >200 nt for each sample to be processed. It is necessary to have RNA molecules >200 nt for efficient library construction and this value for each sample will determine appropriate conditions at various steps in the workflow.

2.6.6 TCR-Seq

One mechanism by which checkpoint inhibitors are thought to elicit their effects by broadening the TCR repertoire. We will perform high-throughput sequencing of the TCR Vb CDR3 regions on flow cytometrically sorted T-cell subsets to assess the effect of immunotherapy on the diversity of the T-cell repertoire and assess for correlation to clinical outcomes. TCR sequencing analysis may be performed using DNA from tumor tissues as well as PBMC. Briefly, 500 ng tumor DNA or 3-6 µg PBMC DNA will be subjected to high throughput TCR Vβ CDR3 sequencing on an Illumina HiSeq sequencer with at least 5-fold coverage by ImmunoSEQ™ sequencing (Adaptive Biotechnologies, Seattle, WA). TCR diversity and clonality (defined as 1-(entropy)/log2(#) of productive unique sequences, where the entropy term takes into account the varying clone frequency) will be calculated using a software by Adaptive Technologies. T-cell repertoire diversity and clonality will be correlated with clinic-pathological parameters such as response to treatment, survival, and immune infiltrating profile, as well as genomic profiles (total mutation burden, non-synonymous mutation burden, predicted neoantigen burden, clonal mutation burden and clonal predicted neoantigen burden). TCR profile generated from treatment-refractory tumors at the time of disease progression will be compared to data from pre-treatment tumor samples to explore the TCR repertoire evolution of these tumors under

therapeutic pressure. The dynamic changes of TCR from PMBC, when longitudinal blood samples are available, will be correlated to response to immune checkpoint blockade or chemotherapy and survival.

2.6.7 Leukemia-specific T-cell response

Although AML cells express antigens recognizable to host T-cells, established leukemia is rarely eradicated by the host T-cells (Teague and Kline, 2013). AML cells employ a number of immune evasion mechanisms, which inhibit the generation or functional execution of anti-tumor immune responses including negative co-stimulatory ligands such as PD-L1 and the expansion and/or induction of suppressive cell types (T_{regs}), myeloid-derived suppressor cells (MDSC). Animal models have shown that blockade of the PD-1 pathway can boost leukemia antigen-specific T-cell responses (Zhang *et al*., 2009). Splenocytes, isolated from WT and PD-$1^{-/-}$ mice 12 days after they were challenged with C1498. GFP AML cells, were exposed to irradiated tumor cells as an antigenic source for T-cell re-stimulation. In IFN-γ ELISpot assays, significantly greater numbers of IFN-γ spot-forming cells were detected from PD-$1^{-/-}$ compared with WT spleens (319209 spots *vs*. 3019 spots, respectively; P=0.001) demonstrating improved anti-leukemia directed T-cell response with interruption of the PD-1 pathway. Similarly, higher numbers of IFN-γ spot-forming cells were detected in spleens of mice treated with the PD-L1 blocking antibody compared with the isotype control antibody (50.1 ±21.1 spots *vs*. 25.9 ±14.9 spots, respectively; P=0.001). Furthermore, survival following AML induction was significantly superior in PD-1-deficient mice as well as WT mice following administration of anti-PD-L1 antibodies compared to WT mice without anti-PD-L1 antibody treatment. These studies have led us to test the question whether the combination of chemotherapy with the anti-PD-1 antibody pembrolizumab (MK-3475) can lead to an improvement in leukemia antigen-specific T-cell response.

Patient monocytes or $CD34^+$ progenitors will be cultured *in vitro* with granulocyte macrophage-colony stimulating factor (GM-CSF) plus interleukin-4 (IL-4) in order to differentiate these cells into DC as described in detail elsewhere (Sallusto and Lanzavecchia, 1994). These DC will then be loaded with and primed by the patients' leukemia cell lysate (Rainone *et al*., 2016). The stimulatory capacity of antigen loaded DCs will be tested by flow cytometric IFN-γ cytokine analysis of the patient's own $CD3^+$ naïve responder cells cultured with the patient's own leukemia lysate pulsed DCs. Brefeldin A will be added to the cultures during the last 6 hours of stimulation to block protein secretion. Cells will be washed in PBS split in different flow cytometry tubes. Following blocking with anti-FCγR to reduce a-specific signals for 15 mins, cells will be then stained with anti-CD3, anti-CD4, anti-CD8 antibodies. Cells will then be fixed, permeabilized, and then stained with mAbs specific for IFN-γ. After a 20-minute incubation at 4°C in the dark, cells will be washed and fixed in 1% paraformaldehyde in PBS.

2.6.8 DNA methylation assays

DNA methylation by HELP (HpaII tiny fragment Enrichment by Ligation-mediated PCR) Genomic DNA will be extracted from the mononuclear cell fraction of bone marrow specimens collected from enrolled patients at baseline, after Cycles 1, 2, 4, 6, at 1 year of treatment, and end of treatment/disease progression using the Puregene kit from Qiagen (Valencia, CA). HELP

representations will be prepared as previously described (Figueroa, 2009) and hybridized onto a custom long-oligonucleotide microarray designed to cover 25,626 HpaII amplifiable fragments (~50,000 unique CpG sites annotated to ~22,000 unique RefSeq genes) (Roche-NimbleGen, design ID: 2006-10- 26_HG17_HELP_Promoter) at the Weill Cornell Medical College Epigenomics Core Facility. Labeling, hybridization, and scanning will be performed as previously described (Figueroa, 2008). Quality control and array normalization were performed following our previously described methods (Figueroa, 2010; Thompson, 2008) and any arrays that did not pass our quality control were excluded from the analysis. In order to correct for the introduction of hybridization batches caused by a technical change in the microarray printing process introduced by NimbleGen while the trial was still ongoing, we subjected each individual channel to batch correction using the ComBat algorithm (Johnson, 2007) and the adjusted channels were then used to calculate the final HpaII/MspI ratio.

2.6.9 Assessment of MRD

2.6.9.1 MRD assessment by multicolor flow cytometry (MFC) as an integral biomarker

Achievement of MRD negativity at complete remission will be the primary endpoint of the trial, however, no therapeutic decisions will be made using the data generated. This application proposes to use multiparameter flow cytometry (MPF) to detect MRD in adults with AML. The results of these determinations will be compared with similar residual disease data generated by dual sequencing, which will be obtained an exploratory biomarker. Patients with AML will have MRD measured in the bone marrow (BM) and peripheral blood (PB) at the time of study entry to determine leukemia-associated immunophenotypic abnormalities and then BM will be evaluated for residual disease and after the fourth cycle of therapy

The central MRD studies will be performed solely in the flow cytometry laboratory located in the Hematopathology Laboratory at the Department of Pathology and Laboratory Medicine Children's Hospital Los Angeles directed by Dr. Brent Wood. This laboratory has more than 15 years of experience performing flow cytometric MRD assays on over 10,000 pediatric patients enrolled on past and ongoing Children's Oncology Group (COG) B-ALL and T-ALL clinical trials. The laboratory also has a strong publication record for the flow cytometric detection of MRD in AML and performs such testing for Southwest Oncology Group (SWOG) trials in adults and for multiple biopharma clinical trials.

The flow cytometry assay proposed for use in this trial is validated and multiple publications support its prognostic relationship to outcome in AML. The assay relies on the principle that immunophenotypic (IP) abnormalities exist in leukemia progenitor cells that distinguish them from normal progenitors (Wood, 2016). Cells are stained with antibodies that have been previously shown to be informative for this purpose, and that have been conjugated to different fluorochromes designed to maximize the resolution between normal and abnormal cells. Specifically, the antibody combinations used are outlined in the following table:

Combination	PB/ BV421	FITC	PE	PE-TR	PE-X	PE-Cy7	A594	APC	APC-A700	APC-H7

AML M1	HLA-DR	CD15	CD33	CD19	CD117	CD13	CD38	CD34	CD71	CD45
AML M2	HLA-DR	CD64	CD123	CD4	CD14	CD13	CD38	CD34	CD16	CD45
AML M4	HLA-DR	CD56	CD7		CD5	CD33	CD38	CD34		CD34

Stained cells are analyzed on a LSRII flow cytometer (Becton Dickinson, San Jose, CA). The proportion of leukemic MRD cells is expressed as a percentage of white cells ($CD45^+$) in each of the tubes. A total of 1,000,000 events is the acquisition target, with 100,000 white cell events being the minimum number of events required. With this number of events it is possible to detect leukemic cells with a routine sensitivity of 0.1%, better in a subset of cases. This assay was used on the SWOG (**S0106**) and other clinical trials. The flow cytometric assay for AML MRD has been used in over 2,000 children and adults with AML tested in our clinical reference laboratory since 2006 and is being used as the assay in multiple biopharma sponsored clinical trials. Precision studies performed in our laboratory on 3 replicates of 3 samples targeted to have 1%; 0.1% and 0.01% MRD *via* serial dilution demonstrated CVs of 3.5%, 6.6% and 6.4%, respectively. The ~7% CV seen with samples with approximately 0.01% MRD supports the interpretation that the sensitivity of the assay is 0.01%.

The current validation of the assay is for a threshold of 0.1% but there is a variable level of sensitivity of the assay in the range of roughly 0.01% - 0.1% depending on leukemic immunophenotype. We will use 0.1% as a threshold for MRD negative disease for purposes of the primary endpoint of the study as per ELN guidelines and using the current validation level threshold of the assay, but we will explore other thresholds such as 0.01% given the ability of the assay to detect this difference in some patients depending on the leukemia immunophenotype and also recognizing that the clinically relevant threshold is not well established yet.

2.6.9.2 MRD assessment by duplex sequencing (DS) as an exploratory biomarker

NGS provides a powerful new platform for MRD detection. Using NGS, millions of DNA fragments can be sequenced in parallel and pieced together to determine the sequence of large regions of the genome. The sensitivity of conventional NGS, however, is limited by a relatively high error rate, which makes it extremely difficult to differentiate sequencing errors from true low-frequency mutations. If the accuracy of NGS could be further improved to meet or exceed that of conventional MRD detection methods, NGS would be an ideal tool for MRD detection because of its reproducible digital nature, generalizability for essentially all AML patients, and ease of implementation in any NGS equipped lab. Furthermore, rather than simply identifying the presence of MRD, NGS would provide genomic information about clonal composition and dynamics, which may improve the predictive value and clinical utility.

Therefore, we want to apply DS to MRD detection in AML as an exploratory endpoint. The sensitivity of DS far surpasses single-stranded tagging methods and can detect mutations at a sensitivity greater than three orders of magnitude compared to NGS methods (Schmitt *et al*., 2012). The error rate of <1 per 10 million base pairs sequenced (the probability of identical "jackpot" errors in the same position on both strands) is achieved by independently sequencing the two strands of each DNA molecule and building double-stranded consensus sequences. As the strands are complementary, true mutations will be found at the same position in both strands.

The Radich Lab is working with TwinStrand (a University of Washington "spin off") to use DS to sequence mutations in acute and chronic leukemia (Araki *et al*., 2016). This team has carefully selected a panel of 25 genes with the most common point mutations, insertions, deletions, fusions, and duplications associated with AML. They have developed probes targeting whole genes (*e.g*., *RUNX1*, *TP53*, *etc*.) or specific mutational hotspots (*e.g*., *FLT3* point mutations and internal tandem duplications (ITD), NPM1 insertions, *etc.*). These genes are covered by a ~40 kilobase (kb) panel which comprises a median of 99% of all AML-associated mutations within the selected genes. A ~30 kb panel of genes will be used. Sequencing to a depth of 20,000x is expected to provide a 98% power to detect an allele frequency of 0.02% (1 in 5000) and 86% power to detect alleles at 0.01% frequency (see Figure 3, below).

De-identified DNA samples will be shipped to TwinStrand for DS at an average molecular depth of 20,000x. Samples will initially be investigator-blinded as to any patient information. Library preparation, sequencing, and analysis will be performed with TwinStrand's optimized workflow, and TwinStrand's bioinformatics core will perform all analyses related to assay output. Dr. Radich's lab, after review of all published AML genomes, has carefully selected a panel of 25 genes with the most common point mutations, insertions, deletions, fusions and duplications associated with AML. They have developed probes targeting whole genes (*e.g*., *RUNX1, TP53, etc*.) or specific mutational hotspots (*e.g*., *FLT3* point mutations and internal tandem duplications [ITD], *NPM1* insertions, *etc*.). These genes are covered by a ~40 kilobase (kb) panel which comprises a median of 99% of all AML-associated mutations within the selected genes.

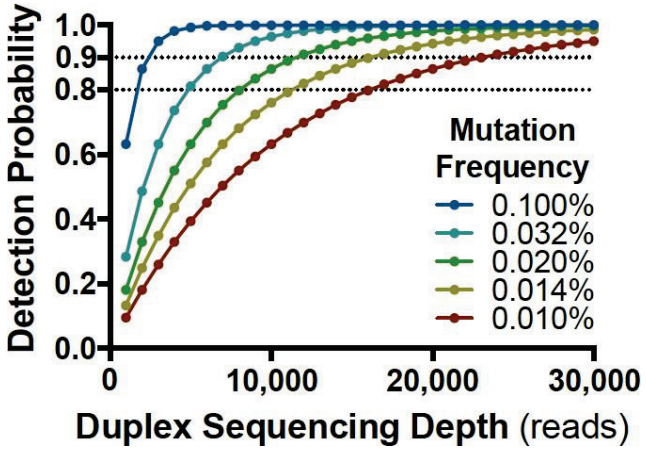


Figure 2. Sensitivity of Duplex Sequencing is a function of input DNA quantity and sequencing depth. Low-level mutations can be detected reliably at 20,000x based on statistical modeling (Unpublished Data).

2.6.9.3 Comparing DS and MFC for MRD detection as an exploratory biomarker

Unfortunately, standard MRD detection methods including MFC and RT-PCR have serious shortcomings in terms of sensitivity, applicability, and reproducibility. Thus, patients without

detectable MRD still relapse. In the SWOG study, **S0106** for newly diagnosed adult AML, MRD status by MFC was a strong predictor of survival, yet MRD predicted relapse-free survival with a poor c-statistic of 0.58 (0.5=no predictive value; 1.0=perfect prediction) (Petersdorf *et al*., 2013). In addition, ~25% of patients without MRD prior to allogenic hematopoietic cell transplant (alloHCT) relapse, while ~65% of those with MRD do not (Araki *et al*., 2016). Furthermore, optimal performance of MFC depends not only on immunophenotype, but also on flow cytometer, panel design, and operator experience. DS is not only more sensitive than MFC but also interpretation is fully digital and "automatic" and does not require manual review by a pathologist or technician and can be easily implemented an any NGS capable lab. Finally, DS will not simply reveal whether or not MRD is present, but the exact mutational nature of the clones comprising residual disease.

In order to compare both approaches, DS will be performed on samples at a molecular depth of 20,000x and the same samples will also be analyzed by 10-color MFC, although researchers will be blinded to MFC results.

2.6.9.4. Application of duplex sequencing strategy for circulating cell-free tumor DNA-based longitudinal minimal residual disease monitoring

While measurable residual disease (MRD) is a well-recognized predictive biomarker for relapse in AML, the current standard of care MRD detection technique - namely multi-parameter flow cytometry (MFC) – does not accurately predict leukemic relapse, due to poor reproducibility and sensitivity, thereby limiting the utility of MFC-based MRD as a reliable surrogate end-point in AML clinical trials. With the growing, unmet need for more accurate MRD approaches in AML studies, the current application seeks to leverage the unprecedented accuracy and sensitivity of the sophisticated duplex sequencing technology to capture very low-level circulating tumor DNA (ctDNA), also known as circulating tumor free DNA (cfDNA). as a strategy to potentially more accurately predict early relapse. The proposed DS-based ctDNA MRD detection approach will not only serve as a proof-of-principle study supporting its utility as a powerful biomarker of relapse in AML but, if shown to be a more accurate predictor of relapse, will lay grounds for its implementation as a potential surrogate marker of long-term response in future AML clinical trials.

For this assay, samples of 8-10mL will be collected at each time point in which MRD is collected from peripheral blood. This collection will be in Streck tubes that are provided to sites. Aside from the different type of tube. these sample will be collected, processed and shipped in the same manner and with the MRD tubes at the different time points. These samples will be sent to Dr. Brent Wood's laboratory where they will be stored, and subsequently sent to Dr. Radich's laboratory where the assays will be conducted.

2.6.10 Gut microbiome characterization

There is increasing evidence that dynamic changes within the microbiome can affect both immune and cancer cells. Imbalance in microbial communities promotes chronic inflammation and predisposes to cancer (Garett *et al*, 2015). Our collaborators have shown that microbiome is also

involved in the metabolism of drugs and could explain person-to-person variability and responsiveness to therapy (Alexander *et al,* 2017, Zimmermann *et al,* 2019). Unlike in several solid tumors wherein, immune-check point blockade is used alone or in combination or with cytotoxic agents, the drug synergism to treat AML is unique. Majority of these combinations results in mucositis which helps microbes breach gastro-intestinal (GI) epithelial barrier. It is plausible that with each cycle of treatment, exposure of immune cells to time -prevalent oral and GI microbiome is subject to temporal variance. Extrapolating preclinical data, taxonomically unique microbes could further determine priming of antigen presenting cell interaction with immune-effector cells that ultimately determine clinical phenotype. Microbial metabolites are also important regulators of immune-responses. Based on our current understanding on the mechanism of action of PD-1 inhibitors, our overall hypothesis is that gastro-intestinal microbiome holds salutary prognostic and therapeutic promise in patients with AML. Our preliminary hypothesis is that gut microbiome and its metabolites modulate clinical responses and toxicity in patients with AML receiving Check Point Inhibitors (CPI). To this end our proposal will test the longitudinal relationships between the taxonomy and diversity of gut microbiome and metabolite on clinical outcomes and toxicity.

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Newly diagnosed and pathologically-confirmed, previously untreated AML as defined by World Health Organization (WHO) criteria. Bone marrow biopsy, or aspirate or peripheral blood that were obtained up to 3 weeks before signing consent are allowed for purposes of confirming AML diagnosis for eligibility purposes. Secondary AML arising from prior myelodysplastic syndrome (MDS), as long as they have not received more than full cycle of hypomethylating agent therapy for MDS,] and t-AML) are also allowed. AML arising from antecedent hematologic disorders defined as prior MDS, MPN, or aplastic anemia are allowed. Note 1: Patients must have evidence of bone marrow involvement on aspirate or biopsy. Patients with only extramedullary disease and no bone marrow involvement will be excluded. Note 2: Every effort should be made to get an aspirate for central flow assessment at screening and all subsequent required time points, but in cases where an aspirate cannot be collected-including dry taps-the patient will not be excluded and assessments will be performed on PB which should be collected at every time that BM is collected. Note 3: Some patients with AML require initiation of therapy quickly after diagnosis, and full metaphase karyotype results in some centers can take 2-3 weeks to result. To avoid this issue being an impediment to accrual to study or to cause delays in initiation of therapy in patients who need fast initiation of therapy, we allow use of karyotype and/or FISH results on samples from blood or marrow that were obtained up to 3 weeks before signing consent for purposes of eligibility and stratification. In any case, results from FISH or karyotype should exclude presence of CBF abnormalities by time of randomization.

3.1.2 Age ≥60 years.

3.1.3 Patients who are ineligible for intensive chemotherapy according to treating physician's assessment or who refuse intensive chemotherapy.

3.1.4 ECOG performance status of 0-3 (See Appendix A).

3.1.5 Prior use of lenalidomide, erythropoiesis-stimulating agents (ESAs), and growth factors is allowed if used to treat prior MDS. AML must be previously untreated except for hydroxyurea, or ATRA for suspicion of APL but both should be discontinued prior to initiation of study therapy. Hypomethylating agents are not allowed to have been used for AML therapy. If hypomethylating agent therapy was used for prior MDS or MPN therapy then it should not have exceeded one full cycle. Note: One dose of prophylactic intrathecal therapy is allowed during or before screening if a lumbar puncture is performed to rule out CNS involvement.

3.1.6 Hydroxyurea, or leukopheresis are allowed for management of hyperleukocytosis, as well as ATRA, before initiation of study therapy. White blood cell (WBC) count must be < 25 $\times 10^9$/L to start on study therapy per venetoclax label. Hydroxyurea and ATRA may be administered up to one day prior to start of study treatment.

3.1.7 Intermediate-risk or poor risk AML as well as favorable risk by NCCN/ELN with the exception of "good-risk" cytogenic profile (*i.e.* for eligibility patient should lack the presence of t(8;21), (inv[16] or t[16;16]), or t(15;17) by full cytogenetics or FISH). Clarification: We allow use of karyotype and/or FISH results (as well as FLT3 results) on samples from blood or marrow that were obtained up to 3 weeks before signing consent for purposes of eligibility and stratification. Adverse karyotype can be determined based on FISH results (e.g. loss of chromosome 7 or 5 or 3 or more abnormalities) based on the specific probes used in the FISH. If results of full metaphase karyotype are not available and the available FISH results do not suggest an adverse karyotype, and there is a need to initiate therapy before those full results are available, then the patient can be stratified into the unknown/intermediate NCCN cytogenetic group for randomization purposes. In any case, results from FISH or karyotype should show that CBF abnormalities are NOT present by at time of randomization as the presence of CBF abnormalities is an exclusion factor.

3.1.8 Patients must have adequate organ function:

System	**Laboratory Value**
Renal	
Creatinine **OR** measured or calculated creatinine clearance (CrCl) [a,b] (See Appendix B)	≤1.5 × ULN **OR** ≥60 mL/min for patient with creatinine levels >1.5 × institutional ULN
Hepatic	

System	Laboratory Value
Total bilirubin	≤1.5 × ULN **OR** Direct bilirubin ≤ULN for patients with total bilirubin levels >1.5 × ULN
AST (SGOT) and ALT (SGPT)	≤3 × ULN **OR** ≤5 × ULN for patients with liver metastases
[a] Creatinine clearance (CrCl) should be calculated per institutional standard. [b] Glomerular filtration rate (GFR) can also be used in place of creatinine or CrCl.	

3.1.9. Patients who are Human Immunodeficiency Virus (HIV) positive may participate IF they meet the following eligibility requirements:

1. They must be stable on their anti-retroviral regimen, and they must be healthy from an HIV perspective.
2. Patients must have an undetectable HIV viral load.

3.1.10 Patients with a known history of hepatitis C virus (HCV) infection must have been treated and cured. For patients with HCV infection who are currently on treatment, they are eligible if they have an undetectable HCV viral load. For patients with evidence of chronic hepatitis B virus (HBV) infection, the HBV viral load must be undetectable on suppressive therapy, if indicated.

3.1.11 Patients who have undergone major surgery must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.

3.1.12 Female patients of childbearing potential must have a negative urine or serum pregnancy test within 72 hours prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required. A female of childbearing potential is any woman, regardless of sexual orientation or whether they have undergone tubal ligation, who meets the following criteria: 1) has not undergone a hysterectomy or bilateral oophorectomy; or 2) has not been naturally postmenopausal for at least 24 consecutive months (*i.e.*, has had menses at any time in the preceding 24 consecutive months).

Female patients of childbearing potential must be willing to use an adequate method of contraception as outlined in Section 6.6 (Contraception and Pregnancy) for the course of the study through 120 days after the last dose of study medication.

Male patients who have a female partner of childbearing potential must agree to use an adequate method of contraception as outlined in Section 6.6 (Contraception and Pregnancy), starting with the first dose of study therapy through 120 days after the last dose of study therapy.

NOTE: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the patient.

3.1.13 Ability to understand and the willingness to sign a written informed consent document. Participants with impaired decision-making capacity (IDMC) who have a legally-authorized representative (LAR) and/or family member available will also be eligible.

3.2. Exclusion Criteria

3.2.1. Patients with CBF-AML and acute promyelocytic leukemia (APL).

3.2.2. Received a prior anti-cancer mAb within 4 weeks prior to study registration or have not recovered (recovery defined as baseline or ≤ grade 1) from AEs due to agents administered more than 4 weeks earlier.

3.2.3. Prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent.

3.2.4. Patients who have had chemotherapy, targeted small molecule therapy (aside from imatinib, dasatinib, or nilotinib, hydroxyurea, or ATRA), or radiotherapy within 4 weeks (6 weeks for nitrosoureas or mitomycin C) prior to entering the study.

3.2.5. Left ventricular ejection fraction <50% as determined by either echocardiogram or MUGA.

3.2.6. Patients who have not recovered from AEs due to prior anti-cancer therapy (*i.e.*, have residual toxicities > grade 1) with the exception of ≤ Grade 2 neuropathy and alopecia.

NOTE: Participants must have recovered from all radiation-related toxicities, not require corticosteroids, and not have had radiation pneumonitis. A 1-week washout is permitted for palliative radiation (≤2 weeks of radiotherapy) to non-CNS disease).

3.2.7. Patients currently participating and receiving study therapy or have participated in a study of an investigational agent and received study therapy or used an investigational device within 4 weeks of the first dose of treatment are ineligible.

3.2.8. History of hypersensitivity to pembrolizumab (MK-3475) or any of its excipients, or other agents used in this study.

3.2.9. Current use of systemic corticosteroids or immunosuppressive agents.
EXCEPTION: Low doses of steroids (<10 mg of prednisone or equivalent dose of other steroid) used for treatment of non-hematologic medical condition (*e.g.*, chronic adrenal insufficiency), inhaled corticosteroids, or topical steroids are permitted.

3.2.10. Other active primary malignancy (other than non-melanomatous skin cancer or carcinoma *in situ* of the cervix) requiring treatment or limiting expected survival to ≤2 years.

NOTE: If there is a history of prior malignancy, they must not be receiving other specific treatment (other than hormonal therapy for their cancer).

3.2.11. Patient with known active CNS disease and/or carcinomatous meningitis before study enrollment. Assessment of the CSF is not required to enroll in the study unless there is clinical suspicion for CNS involvement. However, if CSF assessment is performed for any reason, there should be no evidence of active leukemia in the CSF as per investigator judgement. Up to one dose of prophylactic intrathecal chemotherapy is allowed prior to study enrollment. Subjects with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least four weeks prior to the first dose of protocol treatment and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases, and are not using steroids for at least 7 days prior to protocol treatment. This exception does not include carcinomatous meningitis which is excluded regardless of clinical stability.

3.2.12. Patients who received prior allogenic transplant.

3.2.13. Patient with a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator.

3.2.14. Patient with a diagnosis of immunodeficiency or receiving high dose systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of treatment.

3.2.15. Patient with active autoimmune disease except for patients with hypothyroidism and vitiligo that has required systemic treatment in the past 2 years (*i.e*., with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (*e.g*., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, *etc.*) is not considered a form of systemic treatment.

3.2.16. Patient with a known history of non-infectious pneumonitis that required the use of steroids or current non-infectious pneumonitis

3.2.17. Patient with active uncontrolled infection.

3.2.18. Patient with a known history of active TB (*Bacillus Tuberculosis*).

3.2.19. Patients with uncontrolled intercurrent illness.

3.2.20. Patients with psychiatric illness/social situations that would limit compliance with study requirements

3.2.21. Pregnant women are excluded from this study because pembrolizumab (MK-3475) is humanized antibody with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with pembrolizumab, breastfeeding should be discontinued if the

mother is treated with pembrolizumab. These potential risks may also apply to other agents used in this study

3.2.22. Patients with no bone marrow involvement (*i.e.*, those with only extramedullary disease).

3.2.23. Patients who received prior hypomethylating agent (HMA) therapy for more than one full cycle in treatment for prior MDS. Patient must not have received HMA therapy for treatment of AML.

3.2.24. Patients that received a live vaccine within 30 days of planned start of study therapy.

NOTE: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (*e.g.*, Flu-Mist®) are live attenuated vaccines, and are not allowed.

3.2.25. Patients with active hemolytic anemia requiring immunosuppressive therapy or other pharmacologic treatment. Patients who have a positive Coombs test but no evidence of hemolysis are NOT excluded from participation.

3.3. Inclusion of Women and Minorities

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

4. REGISTRATION PROCEDURES

4.1. Investigator and Research Associate Registration with CTEP

Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all individuals contributing to NCI-sponsored trials to register and to renew their registration annually. To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account at https://ctepcore.nci.nih.gov/iam. In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) (*i.e.*, clinical site staff requiring write access to Oncology Patient Enrollment Network (OPEN), Rave, or acting as a

primary site contact) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) at https://ctepcore.nci.nih.gov/rcr.

RCR utilizes five person registration types.

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

RCR requires the following registration documents:

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

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

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

In addition, all investigators act as the Site-Protocol PI, consenting/treating/drug shipment, or as the CI on the DTL must be rostered at the enrolling site with a participating organization (*i.e.*, Alliance).

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

4.2. Site Registration

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

IRB Approval

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

Sites using their local IRB or REB must submit their approval to the CTSU Regulatory Office using the Regulatory Submission Portal located in the Regulatory section of the CTSU website. Acceptable documentation of local IRB/REB approval includes:

- Local IRB documentation,
- IRB-signed CTSU IRB Certification Form, and/or
- Protocol of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption Form.

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

- Holds an Active CTEP status,
- Rostered at the site on the IRB/REB approval (*applies to US and Canadian sites only*) and on at least one participating roster,
- If using NCI CIRB, rostered on the NCI CIRB Signatory record,
- Includes the IRB number of the IRB providing approval in the Form FDA 1572 in the RCR profile, and
- Holds the appropriate CTEP registration type for the protocol.

Additional Requirements

Additional requirements to obtain an approved site registration status include:

- An active Federalwide Assurance (FWA) number,
- An active roster affiliation with the Lead Protocol Organization (LPO) or a Participating Organization, and
- Compliance with all protocol-specific requirements (PSRs).

4.2.1. Downloading Regulatory Documents

Download the site registration forms from the protocol-specific page located on the CTSU members' website. Permission to view and download this protocol and its supporting documents is restricted based on person and site roster assignment. To participate, the institution and its associated investigators and staff must be associated with the LPO or a Participating Organization on the protocol.

- Log on to the CTSU members' website (https://www.ctsu.org) using your CTEP-IAM username and password,
- Click on *Protocols* in the upper left of your screen
    - Enter the protocol number in the search field at the top of the protocol tree, or
    - Click on the By Lead Organization folder to expand, then select **LAO-CT018** / Yale University Cancer Center LAO, and protocol number 10334
- Click on *Documents*, select *Site Registration*, and download and complete the forms provided. (Note: For sites under the CIRB initiative, IRB data will load automatically to the CTSU as described above.)

4.2.2. Protocol Specific Requirements For 10334 Site Registration

Upon site registration approval in RSS, the enrolling site may access OPEN to complete enrollments. The enrolling site will select their credentialed provider treating the subject in the OPEN credentialing screen, and may need to answer additional questions related to treatment in the eligibility checklist.

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

4.2.3. Submitting Regulatory Documents

Submit required forms and documents to the CTSU Regulatory Office via the Regulatory Submission Portal on the CTSU website.

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

Institutions with patients waiting that are unable to use the Regulatory Submission Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 in order to receive further instruction and support.

Delegation of Tasks Log (DTL)

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

4.2.4. Checking Site Registration Status

You can verify your site's registration status on the members' side of the CTSU website.

- Log on to the CTSU members' website
- Click on *Regulatory* at the top of your screen
- Click on *Site Registration*
- Enter your 5-character CTEP Institution Code and click on Go

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

4.3. Patient Registration

4.3.1. OPEN / IWRS

The Oncology Patient Enrollment Network (OPEN) is a web-based registration system available on a 24/7 basis. OPEN is integrated with CTSU regulatory and roster data and with the Lead Protocol Organization (LPOs) registration/randomization systems or Theradex Interactive Web Response System (IWRS) for retrieval of patient registration/randomization assignment. OPEN

will populate the patient enrollment data in NCI's clinical data management system, Medidata Rave.

Requirements for OPEN access:

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

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

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

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

Note: The OPEN system will provide the site with a printable confirmation of registration and treatment information. Please print this confirmation for your records.

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

4.3.2. OPEN/IWRS User Requirements

OPEN/IWRS users must meet the following requirements:

- Have a valid CTEP-IAM account (*i.e.*, CTEP username and password).
- To enroll patients or request slot reservations: Be on an ETCTN Corresponding or Participating Organization roster with the role of Registrar. Registrars must hold a minimum of an AP registration type.
- To approve slot reservations or access cohort management: Be identified to Theradex as the "Client Admin" for the study.
- Have regulatory approval for the conduct of the study at their site.

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

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

4.3.3. Special Instructions for Patient Enrollment

This Study will use the ETCTN Specimen Tracking System (STS).

- All biospecimens collected for this trial must be submitted using the ETCTN Specimen Tracking System (STS) unless otherwise noted.
- The system is accessed through special Rave user roles: "CRA Specimen Tracking" for data entry at the treating institutions and "Biorepository" for users receiving the specimens for processing and storage at reference labs and the Biorepository.
- Please refer to the Medidata Account Activation and Study Invitation Acceptance link on the CTSU website under the Rave/DQP tab.
- **Important: Failure to complete required fields in STS may result in a delay in sample processing.** Any case reimbursements associated with sample submissions will not be credited if samples requiring STS submission are not logged into STS.

Detailed instructions can be found in Section 5.3

4.3.4. OPEN/IWRS Questions?

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

Theradex has developed a Slot Reservations and Cohort Management User Guide, which is available on the Theradex website: http://www.theradex.com/clinicalTechnologies/?National-Cancer-Institute-NCI-11. This link to the Theradex website is also on the CTSU website OPEN tab. For questions about the use of IWRS for slot reservations, contact the Theradex Helpdesk at 609-619-7862 or Theradex main number 609-799-7580; CTMSSupport@theradex.com.

4.4. General Guidelines

Following registration, patients should begin protocol treatment within 7 days. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Study Coordinator should be notified of cancellations as soon as possible.

5. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

5.1 Summary Table for Specimen Collection

Time Point	Specimen	Send Specimens To:
Baseline		
	• 5mL bone marrow aspirate (mandatory)	Local CLIA labs
	• 10 mL blood in EDTA lavender top vacutainer tube (mandatory) • 10 mL bone marrow aspirate (mandatory)	Brent Wood's lab, Children's Hospital Los Angeles
	• 8-10 mL blood in Streck tube (mandatory)	Radich laboratory, Fred Hutchinson Cancer Research Center
	• 10mL blood in EDTA lavender top vacutainer tube (mandatory) • 1 Bone marrow core biopsy, processed to FFPE block (optional) • 30mL blood in sodium Heparin green top vacutainer tubes (mandatory) • 16 mL bone marrow aspirate in EDTA lavender top vacutainer tube (mandatory) • Skin punch biopsy, processed to FFPE block (mandatory) • Stool (OMNIgene-GUT kit) (mandatory)	EET Biobank
Post-Cycle 1		
	• 10 mL blood in EDTA lavender top vacutainer tube (mandatory) • 10 mL bone marrow aspirate (mandatory)	Brent Wood's lab, Children's Hospital Los Angeles
	• 8-10 mL blood in Streck tube (mandatory)	Radich laboratory, Fred Hutchinson Cancer Research Center
	• Stool (OMNIgene-GUT kit) (mandatory)	EET Biobank
Post-Cycle 2		
	• 10 mL blood in EDTA lavender top vacutainer tube (mandatory) • 10 mL bone marrow aspirate (mandatory)	Brent Wood's lab, Children's Hospital Los Angeles
	• 8-10 mL blood in Streck tube (mandatory)	Radich laboratory, Fred Hutchinson Cancer Research Center
Post-Cycle 4		
	• 10 mL blood in EDTA lavender top vacutainer tube (mandatory) • 10 mL bone marrow aspirate (mandatory)	Brent Wood's lab, Children's Hospital Los Angeles
	• 8-10 mL blood in Streck tube (mandatory)	Radich laboratory, Fred Hutchinson Cancer Research Center

	• 10mL blood in EDTA lavender top vacutainer tube (mandatory) • 30 mL blood in sodium Heparin green top vacutainer tubes (mandatory) • 16 mL bone marrow aspirate in EDTA lavender top vacutainer tube (mandatory) • 1 Bone marrow core biopsy, processed to FFPE block (optional)	EET Biobank
Post-Cycle 6		
	• 10 mL blood in EDTA lavender top vacutainer tube (mandatory) • 10 mL bone marrow aspirate (mandatory)	Brent Wood's lab, Children's Hospital Los Angeles
	• 8-10 mL blood in Streck tube (mandatory)	Radich laboratory, Fred Hutchinson Cancer Research Center
	• 10mL blood in EDTA lavender top vacutainer tube (mandatory) • 30 mL blood in sodium Heparin green top vacutainer tubes (mandatory) • 16mL bone marrow aspirate in EDTA lavender top vacutainer tube (mandatory) • 1 Bone marrow core biopsy, processed to FFPE block (optional)	EET Biobank
Every 3 months during maintenance		
	• 10 mL blood in EDTA lavender top vacutainer tube (optional) • 10 mL bone marrow aspirate (optional)	Brent Wood's lab, Children's Hospital Los Angeles
	• 8-10 mL blood in Streck tube (mandatory)	Radich laboratory, Fred Hutchinson Cancer Research Center
After 1 year of maintenance		
	• 10 mL blood in EDTA lavender top vacutainer tube (mandatory) • 10 mL bone marrow aspirate (mandatory)	Brent Wood's lab, Children's Hospital Los Angeles
	• 8-10 mL blood in Streck tube (mandatory)	Radich laboratory, Fred Hutchinson Cancer Research Center

	• 10mL blood in EDTA lavender top vacutainer tube (mandatory) • 30 mL blood in sodium Heparin green top vacutainer tubes (mandatory) • 16 mL bone marrow aspirate in EDTA lavender top vacutainer tube (mandatory) • 1 Bone marrow core biopsy, processed to FFPE block (optional)	EET Biobank
Relapse		
	• 1 Bone marrow core biopsy, processed to FFPE block (optional) • 10mL blood in EDTA lavender top vacutainer tube (mandatory) • 30 mL blood in sodium Heparin green top vacutainer tubes (mandatory) • 16 mL bone marrow aspirate in EDTA lavender top vacutainer tube (mandatory)	EET Biobank
Progression/End of Treatment		
	• 10 mL blood in EDTA lavender top vacutainer tube (mandatory) • 10 mL bone marrow aspirate (mandatory)	Brent Wood's lab, Children's Hospital Los Angeles
	• 8-10 mL blood in Streck tube (mandatory)	Radich laboratory, Fred Hutchinson Cancer Research Center
	• 30 mL blood in Heparin green top vacutainer tubes (mandatory) • 10mL blood in EDTA lavender top vacutainer tube (mandatory) • 16 mL bone marrow aspirate in EDTA lavender top vacutainer tube (mandatory) • 1 Bone marrow core biopsy, processed to FFPE block (optional)	EET Biobank

Note: All bone marrows performed (aspirate and/or biopsy) should be evaluated for disease status locally at the institution. The bone marrow report must be uploaded to the STS and provided to the EET Biobank, when available.

5.2. Specimen Procurement Kits and Scheduling

5.2.1 Specimen Procurement Kits

Kits for the collection and shipment of stool to the EET Biobank can be ordered online via the Kit Management system: (https://kits.bpc-apps.nchri.org).

Users at the clinical sites will need to set up an account in the Kit Management system and select a specific clinical trial protocol to request a kit. Please note that protocol may include

more than one type of kit. Each user may order two kits per kit type per day (daily max = 6 kits). Kits are shipped ground, so please allow 5-7 days for receipt. A complete list of kit contents for each kit type is located on the Kit Management system website.

It is preferred that the sites maintain 2 stool kits on site and 5 Streck tubes on site to prevent delays of screening for patients who might need to start study therapy quickly.

Institutional supplies must be used for all other specimen collection processing, and shipment, with the exception of Streck tubes which will be supplied to the sites on request. Please request a supply upon site activation. Email lbeppu@fredhutch.org or jradich@fredhutch.org. Additional questions regarding Streck tube supply may be directed to Lan Beppu in Dr. Radich lab at Fred Hutch at 206-667-6966.

Aside from the different type of tube, these DS ctDNA-based longitudinal MRD monitoring samples will be collected, processed, and shipped in the same manner as Dr. Wood's MRD tubes at the different time points listed in Section 5.1 Summary Table for Specimen Collection. These Streck tube samples will be sent directly to Dr. Radich's laboratory where the assays will be conducted.

5.2.2 Scheduling of Specimen Collections to the EET Biobank

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

- Bone marrow core biopsies and skin punch biopsies may be collected and processed to FFPE block any day. FFPE blocks may be shipped Monday through Thursday.
- Fresh blood and bone marrow specimens may be collected and shipped Monday through Friday.
- Stool samples may be collected any day and may be shipped on Monday through Thursday.

5.2.3 Scheduling of Specimen Collections for the Brent Wood's Lab at Children's Hospital Los Angeles

Do not draw specimens on Fridays for delivery on Saturday. One EDTA "BM" tube (10 mL of BM aspirate) and one EDTA "PB" tube (10 mL of peripheral blood) should be shipped overnight with a cold pack (do not allow the samples to freeze).

5.2.4 Scheduling of Specimen Collection for Radich laboratory, Fred Hutchinson Cancer Research Center

Please adhere to the following guidelines when scheduling procedures to collect blood in Streck tubes:

1. We can receive samples Monday-Friday, do not ship samples on Fridays for Saturday receipt. Exceptions can be made on case-by-case basis for Friday shipping but should be discussed in advance of performing screening bone studies with the study chair and approved. In any case, please send notification of shipment to lbeppu@fredhutch.org;

jradich@fredhutch.org; anne.caldwell@yale.edu, or call the lab at Fred Hutch at 206-667-6966.
2. Samples should be shipped within 5 days of collection, ideally no later than 48 hrs after collection.
3. Samples do not need to be refrigerated, it can be kept at ambient temperature
4. Our shipping address is:
Radich Laboratory
Fred Hutchinson Cancer Research Center
1100 Fairview Avenue North
Room D4-385
Seattle, WA 98109
Email: lbeppu@fredhutch.org

5.3 Specimen Tracking System Instructions

5.3.1 Specimen Tracking System Overview and Enrollment Instructions

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

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

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

Immediately following enrollment, the institutional report for the diagnosis (e.g., bone marrow pathology report) under which the patient is being enrolled must be uploaded into Rave. The report must include the collection date, and the IWRS-assigned UPID and patient study ID for this trial. **Important: Remove any personally identifying information, including, but not limited to, the patient's name, date of birth, initials, medical record number, and patient contact information from the institutional pathology report prior to submission.**

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

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

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

5.3.2 Specimen Labeling

5.3.2.1 Blood or Bone Marrow Specimen Labels

Include the following on blood or bone marrow specimens (including whole blood and frozen, processed blood products – like serum and plasma):

- Patient Study ID
- Universal Patient ID (UPID)
- Specimen ID (automatically generated by Rave)
- Time point
- Specimen type (*e.g.*, blood, serum)
- Collection date and time (to be added by hand)
- BM Laterality (*e.g.* Right (R) or Left (L) Side if both submitted) – to be added by hand)

5.3.2.2 Tissue or Skin Punch Biopsy Specimen Labels

Include the following on all tissue specimens or containers (*e.g.*, cassette):

- Patient Study ID
- Universal Patient ID (UPID)
- Specimen ID (automatically generated by Rave)
- Time point
- Specimen type (*e.g.*, Bone Marrow Aspirate, *etc.*)
- BM Core Biopsy Laterality (e.g., Right (R) or Left (L) Side if both submitted) – to be added by hand
- Surgical pathology ID (SPID) number (if applicable)
- Collection date

5.3.2.3 Stool Specimen Labels

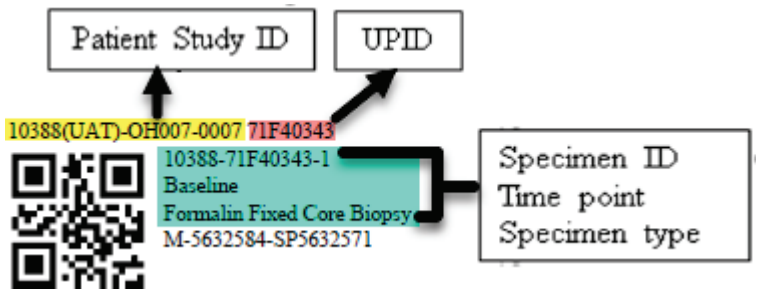
Include the following on all tissue specimens or containers (*e.g.*, cassette):

- Patient Study ID
- Universal Patient ID (UPID)
- Specimen ID (automatically generated by Rave)
- Time point
- Specimen type (*e.g.*, stool etc.)
- Collection date

5.3.2.4 Example of Specimen Label Generated by STS

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

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



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

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

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

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

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

5.3.3 Overview of Process at Treating Site

5.3.3.1 OPEN Registration

All registrations will be performed using the Oncology Patient Enrollment Network (OPEN) system. OPEN communicates automatically with the Interactive Web Response System (IWRS) which handles identifier assignments, any study randomization, and any prescribed slot

assignments. If specimen analysis is required to determine eligibility, the protocol will be setup with multi-step registration.

Registration without eligibility specimen analysis:

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

Any data entry errors made during enrollment should be corrected in Rave.

5.3.3.2 Rave Specimen Tracking Process Steps

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

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

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

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

- Label specimen containers and write collection date and time on each label. For bone marrow samples, also write R for right or L for left on the label.
- After collection, store labeled specimens as described in Section 5.3.2.1.
- Apply an extra specimen label to *each* report before scanning. Return to the **Histology and Disease** form to upload any initial Pathology, Bone Marrow, Radiology, Molecular Reports (up to 4), and Surgical (or Operative) reports. Return to **Specimen Tracking Enrollment** CRF to upload any molecular report (one per specimen) and/or specimen specific pathology or related report (one per specimen) and/or the Tissue Biopsy Verification form (when applicable). Uploaded reports should have protected health information (PHI) data, like name, date of birth, mailing address, medical record number or social security number (SSN), redacted. **Do not redact SPID, block number, diagnosis, or relevant dates, and include the UPID and patient study ID on each document** (either by adding a label or hand-writing).

Step 3: Complete specimen data entry.

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

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

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

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

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

Step 6: Send email notification.

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

Step 7: Ship the specimen(s).

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

5.4 Specimen Collection and Processing

Proper tissue embedding and orientation are necessary in order to support the sample and prevent tissue damage or loss during sectioning (as well as preserve diagnostic histological features). Improperly embedded tissue (e.g., needle cores) can provide incomplete information if diagnostic material is not properly sectioned in a timely manner. Improper orientation of certain samples (e.g., skin tumors) can prevent the evaluation of histological features that may affect survival or recurrence, like depth of tumor invasion and involvement of surgical margins of resection. In order to prevent tissue embedding and orientation errors, refer to the guidelines in Appendix F.

Pre-Analytical Information

Collection site must record all preanalytical information and enter the following into a specimen tracking system (STS) used by each trial network or record and provide with shipping manifest:

1. Time/date blood or tissue sample collection was made as ***Time/Date Specimen Collected***.
2. Ischemia start time (time when sample was devascularized OR estimated time of surgery) – ***Tissue Collection Time/Date***.
3. Ischemic end time ***for each tissue core and surgical segment*** (time when sample was moved to preservative such as formalin or dry ice) – ***Tissue Processing (Formalin Start) Time/Date***.

4. Completion of formalin fixation should be recorded as ***Formalin End Time/Date*** in the STS (or under "comments" if field is not available).
5. Start of 70% Ethanol dehydration should be recorded as ***Ethanol Start Time/Date*** in STS (or under "comments" if field is not available).
6. Time when fixed tissue, held in Ethanol, was placed into an automated processor should be recorded as ***Ethanol End Time/Date*** in the STS (or under "comments" if field is not available).
7. Core # for each core needle biopsy obtained. Each core should be recorded in the STS as a separate specimen with a unique Specimen ID that capture the chronological order in which the biopsy cores were obtained.

5.4.1 Formalin-fixed paraffin-embedded (FFPE) Bone Marrow Core Biopsy Collection and Processing

5.4.1.1 Collection of Bone Marrow Core Biopsy

Bone marrow core biopsies should be collected as per institutional guidelines and standard operating procedures.

5.4.1.2 Processing of Bone Marrow Core Biopsy

Bone marrow core biopsies should be processed to separate FFPE blocks for shipment per institutional guidelines and standard operating procedures. Bone marrow core biopsies should be decalcified in 10% Formic acid, after fixing with neutral buffered-formalin, for 4-6 hours prior to embedding. However, the institutions may follow their respective institutional protocol guidelines if this reagent is not available. **This sample is sent to the EET Biobank.**

1. Neutral-buffered formalin ***must be used*** as fixative (no acid-based products).
2. Samples must be fixed in formalin for ***12-24 hours*** and embedded directly at the collection site. Embedding must be completed ***within 72 hours*** of adding 70% ethanol to tissue.
3. Sites must use automated tissue processors and ***not use*** microwave tissue processors (refer to Appendix G)
4. Sites should follow embedding protocols where the total processing time from 70% ethanol to block embedding ***exceeds 4 hours***.
5. Label the FFPE bone marrow biopsy according to instructions in section 5.3.2.2.

5.4.2 Bone Marrow Aspirate Collection

5.4.2.1 Collection of Bone Marrow Aspirate

1. Label EDTA tubes according to the instructions in section 5.3.2.1.
2. First Syringe: (mandatory) obtain 10 mL of bone marrow aspirate and aliquot into an EDTA tube. Mix the EDTA tubes, by inversion, 8-10 times. Ship this tube on the day of collection (whenever possible) according to instructions in section 5.5. **This tube will be sent to Brent Wood's lab for MRD studies.**

3. Second Syringe (optional): obtain 16 mL of bone marrow aspirate and aliquot 8 mL into each EDTA tube. Mix the EDTA tubes, by inversion, 8-10 times. **This tube will be sent to the EET Biobank for immune correlative studies.**
4. Ship on the day of collection. FedEx Priority Overnight preferred for shipments to the EET Biobank.

**For baseline bone marrow, obtain an additional 5 mL of bone marrow aspirate and aliquot in EDTA tube (to be sent to local CLIA lab) for cytogenetics, next generation sequencing including CBF genetic alterations and FLT3 mutations).

Send all tubes after confirming their specimen labels to the respective laboratories.

5.4.3 Blood Collection

5.4.3.1 Collection of Blood in EDTA Tubes for Shipment to Brent Wood's Lab at Children's Hospital in Los Angeles

1. Label EDTA tubes according to the instructions in section 5.3.1.
2. Collect 10 mL of blood in 2 EDTA tubes, 1 each and gently invert tube 8-10 times to mix.
3. Identify the peripheral blood tube by writing the letters "PB" on the tube.
4. This blood sample **is sent to Brent Wood's Lab at Children's Hospital in Los Angeles** and the biorepository, and can be shipped at ambient temperature.
5. Ship on day of collection (whenever possible) according to instructions in section 5.5.
6. If blood cannot be shipped on the day of collection (*e.g.*, a late scheduled collection), then refrigerate until shipment.

5.4.3.2 Collection of Blood in Streck Tubes for Shipment to Radich laboratory, Fred Hutchinson Cancer Research Center

1. Label Streck tubes according to the instructions in section 5.3.1
2. Collect 8-10 mL of blood in 1 Streck tube and gently invert tube 8-10 times to mix
3. Identify the peripheral blood tube by writing the letters "PB" on the tube.
4. This blood sample **is sent to Radich laboratory, Fred Hutchinson Cancer Research Center** and can be shipped at ambient temperature.
5. Ship on day of collection (whenever possible) according to instructions in section 5.2.4.

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5.4.3.3. Collection of Blood in Green Top Sodium Heparin Tubes for Shipment to the EET Biobank

1. Label Green Top tubes according to the instructions in section 5.3.2.1
2. Collect a total of 30 mL of blood in sodium Heparin tubes and gently invert tube 8-10 times to mix.

3. After collection, gently invert tube(s) 8-10 times to ensure adequate mixing of sodium heparin. Maintain specimens at ambient temperature (room temperature) during collection and transport.
4. Blood should be shipped ambient FedEx Priority Overnight to the EET Biobank the day of collection**.**

5.4.4. FFPE Skin Punch Biopsies

1. Label formalin-filled containers according to instructions in Section 5.3.2.2.
2. Obtain one ≥5mm punch biopsy, and place in cassette. Refer to guidelines in Appendix F to prevent tissue embedding and orientation errors.
3. Snap the cassette lid closed and place cassette into pre-labeled container(s). filled with 10% neutral buffered formalin as soon as possible after collection to prevent air drying. . Samples should be fixed for 24-48 hours.
4. Process and embed following instructions in Appendix G. .Label the FFPE tissue block according to instructions in section 5.3.2.2.
5. Keep FFPE tissue at room temperature until shipment to the EET Biobank.

5.4.5 Collection of Stool in OMNIgene-GUT Tubes for Shipment to the EET Biobank

1. Clinical site staff will explain to patients how to use the kits at the clinic or in the privacy of their home. The collection tube must be labeled by clinical site staff according to instructions in 5.3.2.3 prior to being provided to the patient.
2. Baseline and post Cycle 1 timepoints will use OMNIgene GUT kits (OMR-200.100—shipped ambient) which include a DNA stabilizing solution.
3. Collection kits will include directions for specimen collection and shipment as well as collection and shipping supplies.
4. The patient will collect the stool sample and return the sample to site staff. It is recommended that patients return the specimens within 24 hours, when collected at home. The stool specimen should be kept ambient until shipment.
5. The stool sample and a shipping manifest should be shipped on day of collection (whenever possible) according to instructions provided in the kit and in section 5.6.2.

5.5 Shipping Specimens from Clinical Site to the EET Biobank.

General Shipping Information

Fresh bone marrow aspirate and fresh blood should be shipped ambient. Formalin-fixed paraffin-embedded (FFPE) tissue, including bone marrow biopsy and skin punch specimens are also shipped at ambient temperature. In winter months, please include extra insulation, such as bubble wrap, around the specimen(s) inside the shipping container.

Required Forms for Specimen Submissions:

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

Tissue	Required Forms
Bone Marrow Aspirate or Biopsy	1. Shipping List 2. Corresponding Bone Marrow Report
Other (blood, blood product, stool, etc.)	1. Shipping List

Specimen Shipping Instructions

FFPE tissue may be shipped on Monday through Thursday.

Fresh blood or bone marrow aspirate may be shipped on Monday through Friday. Please select "Saturday Delivery" when shipping fresh blood on a Friday.

Shipping FFPE Blocks in Your Own Container

1. Before packaging blocks, verify that each specimen is labeled according to Section 5.3.2.2.
2. Blocks should be placed in a hard-sided container, preferably a special block holder, to protect the specimen.
3. Place the blocks in a reinforced cardboard shipping box with appropriate packaging filler to minimize movement of specimens within the shipping box.
4. Include a copy of the forms listed above and a shipping manifest from the Specimen Tracking System with each shipment.
5. Please include a cold pack when shipping on hot days and extra insulation on cold days.
6. Ship specimens to the address listed below. FedEx Priority Overnight is strongly recommended to prevent delays in package receipt.

Shipping Blood and Bone Marrow in Your Own Container

1 Before packaging specimens, verify that the collection tube is labeled according to instructions in section 5.3.2.
2 Place the blood collection tube into a zip-lock bag.
3 Place zip-lock bag into a biohazard envelope with absorbent material. Expel as much air as possible and seal the envelope securely.
4 Place the biohazard envelope into a Tyvek envelope. Expel as much air as possible and seal securely.
5 Place the specimen(s) and a copy of the shipping manifest into a sturdy shipping container. In winter months, please use an insulated container and include extra

insulation, such as bubble wrap, inside the shipping container to prevent specimens from freezing.
6 Close the container and tape shut.
7 Attach a shipping label to the top of the shipping container.
8 Attach an Exempt Human Specimen sticker to the side of the container. Add a label indicating "CIMAC specimen" with collection date and time on the outside of the shipping container.
9 Ship specimens via overnight courier to the address below. FedEx Priority Overnight is strongly recommended to prevent delays in package receipt.

Shipping Stool in an Ambient Shipper

The clinical site will ship each specimen to the Biobank. The OMNIgene GUT collected samples can remain at ambient temperatures for a maximum of 60 days but should be shipped as soon as possible after collection.

Detailed instructions for packaging and shipping the stool samples are provided in the kit. Shipping Address

Ship to the address below. Ship formalin-fixed and fresh blood specimens the same day of specimen collection. Do not ship specimens the day before a holiday.

EET Biobank
2200 International Street
Columbus, OH 43228
PH: (614) 722-3270
FAX: (614) 722-2856
Email: BPCMGLab@nationwidechildrens.org

FedEx Priority Overnight service is very strongly preferred.

NOTE: The EET Biobank FedEx Account will not be provided to submitting institutions. There is no central Courier account for this study. Sites are responsible for all costs for shipments to the EET Biobank.

Contact Information for Assistance

For all queries, please use the contact information below:

EET Biobank
Phone: (614) 722-3270
E-mail: BPCMGLab@nationwidechildrens.org

5.6 Shipping of Specimens from Clinical Site to Brent Wood's Lab, Department of Pathology and Laboratory Medicine, Children's Hospital, Los Angeles

5.6.1. Shipping of Specimens to Brent Wood's Lab, Department of Pathology and Laboratory Medicine, Children's Hospital, Los Angeles

5.6.1.1. Specimen Shipping Instructions

1. Specimen should be received by Department of Pathology and Laboratory Medicine within 48 hours of collection.
2. Specimen must be placed in a leak proof vacutainer.
3. Multiple fragile vacutainers must be individually wrapped or separated to prevent contact.
4. The vacutainer must be placed into a leak proof Ziploc biohazard bag in such a way that under normal conditions of transport, they cannot break or leak.
5. The Ziploc bag must be labeled with the universal biohazard symbol.
6. Absorbent material such as absorbent pads or pillows must be placed in the Ziploc bag with sufficient capacity to absorb the entire contents of the vacutainer.
7. The Ziploc bag must be placed into a rigid outer package with suitable cushioning materials.
8. The outer packaging must be clearly and durably marked with the words "Diagnostic Specimen" and 2-inch diamond with "UN3373" inside of the diamond.
9. The outer packaging must be marked with the name, address, and phone number of both sender and recipient.
10. Specimen should be shipped with a cold pack (do not allow to freeze).

The CHLA Department of Pathology and Laboratory Medicine is open Monday-Saturday 8:00- 4:30. As the hospital loading dock is closed on Saturdays, for Saturday delivery to be successful, please:
1. Add on shipping label: "Friday shipping for Saturday delivery. Deliver to Front Desk, Children's Hospital Los Angeles."
2. Notify the CHLA laboratory at plmclinicalimmunologylaboratory@chla.usc; bwood@chla.usc.edu and cc amer.zeidan@yale.edu; anne.caldwell@yale.edu.
3. Provide CHLA the FedEx tracking number and subject number so the lab can track the sample shipment.

5.6.1.2. Shipping Address
Send FedEx tracking number to plmclinicalimmunologylaboratory@chla.usc.edu.

Send the specimens FedEx Priority Overnight to:

Brent Wood, MD, PhD
Department of Pathology and Laboratory Medicine Children's Hospital Los Angeles 4650 Sunset Blvd.
Duque Bldg., 2nd Floor, Room 2-290Los Angeles, CA 90027 Phone: (877) 543-9522

An STS Shipping Manifest Form must be generated and shipped with all specimen submissions.

5.7. Contact Information for AssistanceBiomarker Plan

List of Biomarker Assays in Order of Priority

Note for participating sites: Please see Section 5.1 for details on specimens to collect. The specimens tested are not always the same specimens that are submitted by the site, as processing of blood and tissue will occur at the Biobank prior to testing

Priority	Biomarker Name	Assay (CLIA: Y/N)	Use in the Trial and Purpose	Specimens Tested	Collection Time Points	Mandatory or Optional	Assay Laboratory and Lab PI
Core Biopsy Biomarkers							
1	Multiplex Immunofluorescence	Multiplex IF CLIA: **N**	Exploratory Correlate with clinical response	FFPE Bone Marrow Core Biopsy	Baseline Post cycle 4 Post cycle 6 After 1 year Relapse End of treatment/progression	O	CIMAC Ignacio Wistuba iiwistuba@mdanderson.org
Bone Marrow Aspirate Biomarkers							
1	FLT3 mutations	FLT3 PCR or NGS Panel CLIA: **Y**	Integral Stratification factor	Bone marrow aspirate	Baseline	M	Local CLIA labs
2	Karyotype	Conventional cytogenetics and AML FISH translocation panel CLIA: **Y**	Integral Stratification factor	Bone marrow aspirate	Baseline	M	Local CLIA labs

Priority	Biomarker Name	Assay (CLIA: Y/N)	Use in the Trial and Purpose	Specimens Tested	Collection Time Points	Mandatory or Optional	Assay Laboratory and Lab PI
3	MRD Status	Multicolor flow cytometry CLIA: **Y**	Integral Primary endpoint	Bone marrow aspirate	Baseline Post-cycle 1 Post-cycle 2 Post-cycle 4 Post-cycle 6 Every 3 months during maintenance After 1 year End of treatment/progression	M (O during maintenance)	Brent Wood's Lab, Children's Hospital, Los Angeles
4	MRD Status	Duplex sequencing CLIA: **N**	Exploratory Exploratory endpoint	Bone marrow aspirate (same as #3)	Baseline Post-cycle 1 Post-cycle 2 Post-cycle 4 Post-cycle 6 Every 3 months during maintenance After 1 year End of treatment/progression	M (O during maintenance phase)	Fred Hutchinson Cancer Research Center (shipped from Brent Wood's lab) Jerald Radich jradich@fhcrc.org
5	Immune cell subset analysis	CyTOF CLIA: **N**	Exploratory Correlate with clinical response	Bone marrow aspirate in EDTA (mononuclear cells)	Baseline Post-cycle 4 Post-cycle 6 After 1 year Relapse End of treatment/progression	O	CIMAC Ignacio Wistuba iiwistuba@mdanderson.org
6	Olink (cytokine panel)	Olink CLIA: **N**	Exploratory Correlate with clinical response	Bone marrow aspirate in EDTA (plasma)	Baseline Post-cycle 4 Post-cycle 6 After 1 year Relapse End of treatment/progression	O	CIMAC Sacha Gnjatic Sacha.gnjatic@mssm.edu

Priority	Biomarker Name	Assay (CLIA: Y/N)	Use in the Trial and Purpose	Specimens Tested	Collection Time Points	Mandatory or Optional	Assay Laboratory and Lab PI
7	Whole exome sequencing	WES (tumor) CLIA: **N**	Exploratory Correlate with clinical response	DNA from marrow aspirate (EDTA)	Baseline Relapse End of treatment/progression	O	CIMAC Ignacio Wistuba iiwistuba@mdanderson.org
		WES (germline) CLIA:N	Exploratory Correlate with clinical response	DNA from FFPE skin punch biopsies	Baseline	O	CIMAC Ignacio Wistuba iiwistuba@mdanderson.org
8	RNA-Seq	RNA-Seq CLIA: **N**	Exploratory Correlate with clinical response	RNA from bone marrow aspirate in EDTA	Baseline Relapse End of treatment/progression	O	CIMAC Ignacio Wistuba iiwistuba@mdanderson.org
9	TCR-seq	TCR-Seq CLIA: **N**	Exploratory Correlate with clinical response	DNA from Bone marrow aspirate in EDTA	Baseline Post-cycle 4 Post-cycle 6 After 1 year Relapse End of treatment/progression	O	CIMAC working with Adaptive Ignacio Wistuba iiwistuba@mdanderson.org
10	Leukemia-specific T cell response	Flow cytometry CLIA: **N**	Exploratory Correlate with clinical response	Mononuclear cells from bone marrow aspirate in EDTA tubes	Baseline Post-cycle 4 Post-cycle 6 After 1 year Relapse End of treatment/progression	O	MD Anderson Cancer Center (Non-CIMAC) Gheath Al-Atrash galatras@mdanderson.org
11	DNA methylation assay	Microarray CLIA: **N**	Exploratory Correlate with clinical response	Mononuclear cells from Bone marrow aspirate in EDTA tubes	Baseline Post-cycle 4 Post-cycle 6 After 1 year Relapse End of treatment/progression	O	MD Anderson Cancer Center (Non-CIMAC) Gheath Al-Atrash galatras@mdanderson.org

Priority	Biomarker Name	Assay (CLIA: Y/N)	Use in the Trial and Purpose	Specimens Tested	Collection Time Points	Mandatory or Optional	Assay Laboratory and Lab PI
Blood-based Biomarkers							
1	MRD Status	Duplex sequencing CLIA: **N**	Exploratory Exploratory endpoint	Peripheral blood in EDTA tubes	Baseline Post-cycle 1 Post-cycle 2 Post-cycle 4 Post-cycle 6 Every 3 months during maintenance After 1 year End of treatment/progression	M (O during Maintenance)	Fred Hutchinson Cancer Research Center (shipped from Brent Wood's lab) Jerald Radich jradich@fhcrc.org
2	Immune cell subset analysis	CyTOF CLIA: **N**	Exploratory Correlate with clinical response	PBMCs from blood collected in sodium heparin tubes	Baseline Post-cycle 4 Post-cycle 6 After 1 year Relapse End of treatment/progression	M	CIMAC Ignacio Wistuba iiwistuba@mdanderson.org
3	Olink (cytokine panel)	Olink CLIA: **N**	Exploratory Correlate with clinical response	Plasma (from blood collected in sodium heparin tubes)	Baseline Post-cycle 4 Post-cycle 6 After 1 year Relapse End of treatment/progression	M	CIMAC Sacha Gnjatic Sacha.gnjatic@mssm.edu
4	TCR-seq	TCR-Seq CLIA: **N**	Exploratory Correlate with clinical response	DNA from PBMCs from blood collected in EDTA or sodium heparin tubes	Baseline Post-cycle 4 Post-cycle 6 After 1 year Relapse End of treatment/progression	M	CIMAC working with Adaptive Ignacio Wistuba iiwistuba@mdanderson.org

4	Leukemia-specific T cell response	Flow cytometry CLIA: **N**	Exploratory Correlate with clinical response	Mononuclear cells from Peripheral blood in EDTA tubes	Baseline Post-cycle 4 Post-cycle 6 After 1 year Relapse End of treatment/progression	M	MD Anderson Cancer Center (Non-CIMAC) Gheath Al-Atrash galatras@mdanderson.org

Priority	**Biomarker Name**	**Assay (CLIA: Y/N)**	**Use in the Trial and Purpose**	**Specimens Tested**	**Collection Time Points**	**Mandatory or Optional**	**Assay Laboratory and Lab PI**
5	DNA methylation assay	Microarray CLIA: **N**	Exploratory Correlate with clinical response	Mononuclear cells from Peripheral blood in EDTA tubes	Baseline Post-cycle 4 Post-cycle 6 After 1 year Relapse End of treatment/progression	M	MD Anderson Cancer Center (Non-CIMAC) Gheath Al-Atrash galatras@mdanderson.org
6.	cfDNA	Duplex Sequencing (DS) CLIA: N	Exploratory Exploratory Endpoint	Peripheral blood (Streck)	Baseline Post-cycle 1 Post-cycle 2 Post-cycle 4 Post-cycle 6 Every 3 months during maintenance After 1 year End of treatment/progression	M	To be shipped to Radich laboratory, Fred Hutchinson Cancer Research Center. Molecular Oncology Laboratory at Fred Hutchinson Cancer Research Center Jerald Radich jradich@fhcrc.org
Stool-based biomarkers							
1	Gut microbiome characterization	16sRNA V4 region sequencing of bacterial genomic DNA	• Exploratory Correlate with clinical response	Stool from OMNIgene GUT sample collection kit	• Baseline • Post cycle 1	M	MDACC CIMAC Gheath Al-Atrash galatras@mdanderson.org

*** CyTOF, WES, RNAseq, TCR and Olink analyses in bone marrow will be performed using cells and plasma from a single BM aspirate.**

5.8. Integral Laboratory Studies

5.8.1 *FLT3* mutations

5.8.1.1 Specimen Receipt and Processing at Local Labs

Samples should be received and processed as per standard operating procedures.

5.8.1.2 Site Performing Correlative Study

This study will be conducted by local labs.

5.8.2 Karyotype/FISH

5.8.2.1 Specimen Receipt and Processing at Local Labs

Samples should be received and processed as per standard operating procedures.

5.8.2.2 Site Performing Correlative Study

This study will be conducted by local labs.

5.8.3 MRD Status by Multicolor Flow Cytometry

5.8.3.1 Specimen Receipt and Processing at Brent Wood's Lab, Children's Hospital, Los Angeles

Bone marrow aspirate will be processed as per laboratory standard operating procedures.

5.8.3.2 Site(s) Performing Correlative Study

The study will be performed in the laboratory of Dr. Brent Wood, Children's Hospital, Los Angeles

5.9 Exploratory Correlative Studies

5.9.1 Multiplex Immunofloresence:

5.9.1.1 Specimen(s) Receipt and Processing at the EET Biobank

The EET Biobank will receive and barcode FFPE bone marrow biopsy blocks, and will store them at room temperature until distribution. The EET Biobank will ship the FFPE bone biopsy core tissue from all time points to MDACC CIMAC.

UT MD Anderson Cancer Center
Life Science Plaza Building
2130 W. Holcombe Blvd, LSP9.3003
Houston, TX 77030

c/o Mei Jiang
Mjiang1@mdanderson.org
Phone: 713-563-0373

5.9.1.2 Site(s) Performing Correlative Study

This assay will be performed at MDACC CIMAC in the laboratory of Dr. Ignacio I Wistuba (Email: iiwistuba@mdanderson.org).

5.9.2 RNA-Seq

5.9.2.1 Specimen(s) Receipt and Processing at the EET Biobank

Upon receiving the bone marrow aspirate in EDTA tubes, the EET Biobank will ***pool all samples*** from one timepoint together and prepare Plasma and mononuclear cells following CIMAC guidelines. Plasma aliquots will be stored in a -80°C freezer, and mononuclear cells will be slow-frozen in freezing media and stored in a liquid nitrogen vapor phase freezer.

DNA and RNA will be co-extracted from bone marrow mononuclear cell. An aliquot of RNA from the bone marrow mononuclear cell will be used for this assay.
The EET Biobank will ship this sample to MDACC CIMAC.

UT MD Anderson Cancer Center
Room 4SCR5.2085
1881 East Rd.
Houston, TX 77054
c/o Habibul Islam
MHIslam@mdanderson.org
Phone: 713-794-1068

5.9.2.2 Site(s) Performing Correlative Study

This assay will be performed at MDACC CIMAC in the laboratory of Dr. Ignacio I Wistuba (Email: iiwistuba@mdanderson.org) (shipping address above).

5.9.3 MRD Status by Duplex Sequencing

5.9.3.1 Specimen Receipt and Processing at Brent Wood's Lab, Department of Pathology and Laboratory Medicine Children's Hospital Los Angeles

Bone marrow aspirate and blood will be processed as per laboratory standard operating procedures.

5.9.3.2 Site(s) Performing Correlative Study

The study will be performed in the laboratory of Dr. Jerald Radich at the Fred Hutchinson Cancer Research Center.

5.9.4 Immune cell subset analysis by CyTOF

5.9.4.1 Specimen(s) Receipt and Processing at the EET Biobank

The Biorepository will receive fresh BM aspirate collected in EDTA tubes and blood samples collected in heparin tubes. Upon receiving the bone marrow aspirate in EDTA tubes from the collection site, the EET Biobank will ***pool all samples*** from one timepoint together and prepare plasma and mononuclear cells following CIMAC guidelines. Blood collected at each time point will also be pooled and processed for plasma and mononuclear cells following CIMAC guidelines. Plasma aliquots will be stored in a -80°C freezer, and mononuclear cells will be slow-frozen in freezing media and stored in a liquid nitrogen vapor phase freezer.

The EET Biobank will ship this sample to MDACC CIMAC.

UT MD Anderson Cancer Center
Room 2SCR2.3219
7435 Fannin St
Houston, TX 77054
713-745-6999
c/o Karen Millerchip
kamillerchip@mdanderson.org

5.9.4.2 Site(s) Performing Correlative Study

This assay will be performed at MDACC CIMAC in the laboratory of Dr. Ignacio I Wistuba (Email: iiwistuba@mdanderson.org).

5.9.5 O-link Cytokine panel

5.9.5.1 Specimen(s) Receipt and Processing at the EET Biobank

The Biorepository will receive fresh BM aspirate collected in EDTA tubes and blood samples collected in heparin tubes. Upon receiving the bone marrow aspirate in EDTA tubes from the

collection site, the EET Biobank will ***pool all samples*** from one timepoint together and prepare plasma and mononuclear cells following CIMAC guidelines. Blood collected at each time point will also be pooled and processed for plasma and mononuclear cells following CIMAC guidelines. Plasma aliquots will be stored in a -80°C freezer, and mononuclear cells will be slow-frozen in freezing media and stored in a liquid nitrogen vapor phase freezer.

The EET Biobank will ship this frozen plasma to the Mt. Sinai CIMAC.

Hess Center for Science and Medicine
5th floor, rooms 310/313
Human Immune Monitoring Center (HIMC)
Icahn School of Medicine at Mount Sinai
1470 Madison Avenue
New York, NY 10029

5.9.5.2 Site(s) Performing Correlative Study

This study will be performed at the Mount Sinai CIMAC by Sacha Gnajtic. Email: Sacha.gnjatic@mssm.edu

5.9.6 Whole exome sequencing

5.9.6.1 Specimen(s) Receipt and Processing at the EET Biobank

Upon receiving the bone marrow aspirate in EDTA tubes from the collection site, the EET Biobank will ***pool all samples*** from one timepoint together and prepare Plasma and mononuclear cells following CIMAC guidelines.
Plasma aliquots will be stored in a -80°C freezer, and mononuclear cells will be slow-frozen in freezing media and stored in a liquid nitrogen vapor phase freezer.

DNA and RNA will be co-extracted from bone marrow mononuclear cells. An aliquot of DNA will be used for this assay.

Germline DNA will be extracted from skin punch tissue.

The EET Biobank will ship this sample to MDACC CIMAC.

UT MD Anderson Cancer Center
Room 4SCR5.2085
1881 East Rd.
Houston, TX 77054
c/o Habibul Islam
MHIslam@mdanderson.org
Phone: 713-794-1068

5.9.6.2 Specimen(s) Receipt and Processing at the EET Biobank for FFPE skin punch biopsies

Upon receiving of the skin punch biopsies from the collection site, the EET Biobank will process according to section 5.4.4 Germline DNA will be extracted from FFPE skin punch biopsies.

The EET Biobank will ship this sample to MDACC CIMAC.

UT MD Anderson Cancer Center
Room 4SCR5.2085
1881 East Rd.
Houston, TX 77054
c/o Habibul Islam
MHIslam@mdanderson.org
Phone: 713-794-1068

5.9.6.3 Site(s) Performing Correlative Study

This assay will be performed at MDACC CIMAC in the laboratory of Dr. Ignacio I Wistuba (Email: iiwistuba@mdanderson.org).

5.9.7 TCR-Seq

5.9.7.1 Specimen(s) Receipt and Processing at the EET Biobank

Upon receiving the bone marrow aspirate in EDTA tubes from the collection site, the EET Biobank will ***pool all samples*** from one timepoint together and prepare Plasma and mononuclear cells following CIMAC guidelines. Plasma aliquots will be stored in a -80°C freezer, and mononuclear cells will be stored in liquid nitrogen vapor phase.

Additionally, PBMCs from peripheral blood samples collected in EDTA or heparin tubes will also be used for TCRseq.

DNA and RNA will be co-extracted from bone marrow mononuclear cells and peripheral blood mononuclear cells. An aliquot of DNA will be used for each assay (i.e., for TCRseq in bone marrow aspirate and for TCRseq in peripheral blood).
.
The EET Biobank will ship the samples to Adaptive Biotechnologies, who will work with MDACC CIMAC.

5.9.7.2 Site(s) Performing Correlative Study

This assay will be performed at Adaptive Biotechnologies, which will work with MDACC CIMAC (PI: Dr. Ignacio I Wistuba, Email: iiwistuba@mdanderson.org).

5.9.8 Leukemia-Specific T cell response

5.9.8.1 Specimen(s) Receipt and Processing at the EET Biobank

Bone marrow aspirate and blood will be processed as per laboratory standard operating procedures. The Biorepository will receive fresh BM aspirate collected in EDTA tubes and blood samples collected in EDTA tubes. Upon receiving the bone marrow aspirate in EDTA tubes from the collection site, the EET Biobank will ***pool all samples*** from one timepoint together and prepare plasma and mononuclear cells following CIMAC guidelines. Blood collected at each time point will also be pooled and processed for plasma and mononuclear cells following CIMAC guidelines. Plasma aliquots will be stored in a -80°C freezer, and mononuclear cells will be slow-frozen in freezing media and stored in a liquid nitrogen vapor phase freezer.

The EET Biobank will ship this sample to MD Anderson Cancer Center.

Attn: Lisa St. John, PhD
MD Anderson Cancer Center
Hematopoietic Biology and Malignancy
2SCR4.2215
7435 Fannin St.
Houston, TX 77054

5.9.8.2 Site(s) Performing Correlative Study

This assay will be performed at MD Anderson Cancer Center in the laboratory of Gheath Al-Atrash (Email: galatras@mdanderson.org)

5.9.9 DNA methylation assays

5.9.9.1 Specimen(s) Receipt and Processing at the EET Biobank

Bone marrow aspirate and blood will be processed as per laboratory standard operating procedures. The Biorepository will receive fresh BM aspirate collected in EDTA tubes and blood samples collected in the EDTA tubes. Upon receiving the bone marrow aspirate in EDTA tubes from the collection site, the EET Biobank will ***pool all samples*** from one timepoint together and prepare plasma and mononuclear cells following CIMAC guidelines. Blood collected at each time point will also be pooled and processed for plasma and mononuclear cells following CIMAC guidelines. Plasma aliquots will be stored in a -80°C freezer, and mononuclear cells will slow-frozen in freezing media and be stored in a liquid nitrogen vapor phase freezer.

The EET Biobank will ship this sample to MD Anderson Cancer Center.

Attn: Beatriz Sanchez-Espiridion
Institutional Tissue Bank (ITB)
1515 Holcombe Blvd, Rm G1.3586
Houston, TX 7703
Phone: 713-745-7047
Email: bsanchez2@mdanderson.org

5.9.9.2 Site(s) Performing Correlative Study

This assay will be performed at MD Anderson Cancer Center in the laboratory of Gheath Al-Atrash (Email: galatras@mdanderson.org)

5.9.10 Gut microbiome

5.9.10.1 Specimen(s) receipt and processing at MDACC

Upon receipt of the sample in the OMNIgene-Gut kits, the stool sample will be processed as per laboratory standard operating procedures. Stool aliquots will be stored in a -80°C freezer until distribution.

The EET Biobank will ship this sample to the MDACC CIMAC lab.

UT MD Anderson Cancer Center
4SCRB5.2085, Unit 1954
1881 East Rd.
South Campus Research Building 3
Houston, TX 77054
c/o Chia-Chi (Tina) Chang
CChang2@mdanderson.org

5.9.10.2. Site(s) Performing Correlative Study

The microbiome assay will be performed at MDACC CIMAC in the laboratory of Gheath Al-Atrash (Email: galatras@mdanderson.org.

6. TREATMENT PLAN

6.1 Agent Administration

Treatment will be administered on an inpatient or outpatient basis. Reported adverse events and potential risks are described in Section 10. Appropriate dose modifications are described in Section 7. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

6.1.1 Induction therapy phase
Eligible patients will be randomized before proceeding to AZA + VEN or within one week of starting AZA + VEN in 1:1 ratio to AZA + VEN alone or AZA + VEN + pembrolizumab. As shipment of pembrolizumab to site can take up to 4 business days and can only be ordered after randomization, every effort should be made to randomize the patient as soon as possible. If patient is randomized after initiation of Aza+Ven therapy, the patient should continue to meet all eligibility

criteria up to randomization date. Planned stratification factors include 1) cytogenetics (intermediate/unknown *vs.* adverse karyotype) 2) antecedent hematologic disorder defined as prior MDS, MPN, or aplastic anemia (present *vs.* absent); and 3) reason to not receive intensive chemotherapy (ineligibility *vs* refusal). All patients will receive azacitidine at 75 mg/m^2 IV over 10-40 minutes or SQ x 7 days on Days 1-7 or Days 5-2-2 (to avoid weekend administration) and, including a ramp-up phase to 400 mg /day venetoclax on Days 1-4. Venetoclax will be administered at 400 mg/day (or as dose adjusted for concomitant therapy) on the remaining days of the cycle for the first cycle and on Days 1-21 or 1-28 for subsequent cycles (depending on count recovery). Half of the patients will receive pembrolizumab intravenously starting on Day 8 of the first cycle and then q 3 weeks through cycles 2-6 (intervention arm). The cycles of pembrolizumab run independently from aza+ve therapy. The other half will not receive pembrolizumab (control arm). At time of count recovery (approximately 28-42 days following administration of AZA+VEN), patients will undergo a bone marrow aspirate/biopsy in order to assess for response and MRD. In addition, following count recovery after Cycles 2, 4, and 6, a bone marrow biopsy will be performed to assess for response and MRD status. Patients will be allowed to continue on study as long as the investigator believes they are deriving clinical benefit and not experiencing prohibitive toxicity.

Induction Therapy Regimen Description					
Agent	**Premedications ; Precautions**	**Dose***	**Route#**	**Schedule**	**Cycle Length**
Pembrolizumab (for intervention arm only)	Premedication with antipyretic and antihistamine may be considered. No premedication with steroids	200 mg See Section 8.1.1. for instructions on the preparation of the infusion solution.	IV over 30 minutes (between 25-40 minutes) using an infusion set containing a low-protein binding 0.2 to 5 µm in-line filter. See Section 8.1 for compatible infusion set materials including in-line filter.	Day 8 (Q3W ±3 days) <u>continuous from Day 8 of induction</u>	28 days

Induction Therapy Regimen Description					
Agent	**Premedications ; Precautions**	**Dose***	**Route#**	**Schedule**	**Cycle Length**
Azacitidine	IV formulation is incompatible with 5% dextrose, Hespan, or bicarbonate solutions. Patients should be pre-medicated for nausea and vomiting.	75 mg/m^2	IV over 10-40 minutes or SQ Refer to Section 8.2.1 for additional information.	Days 1-7 or days 5-2-2	
Venetoclax	None.	400 mg (refer to ramp up dosing scheme in Table below) Refer to Section 8.2.2 for additional information.	P.O.	Days 1-28 for Cycle 1, Days 1-21 or 1-28 for subsequent Cycles	
*Dose rounding per institutional guidelines is allowed. #Order should be pembrolizumab, followed by azacitidine, then venetoclax on days when multiple medications are given.					

The patient will be requested to maintain a medication diary of each dose of venetoclax. The medication diary will be returned to clinic staff at the end of each course (see Appendix E).

The following ramp-up scheme will be utilized for venetoclax:

Administer the venetoclax dose according to a daily ramp-up schedule over 4 days to the recommended daily dose of 400 mg as shown in Dosing Scheme for the Ramp-up Phase below. Instruct patients to take venetoclax tablets with a meal and water at approximately the same time each day. Venetoclax tablets should be swallowed whole and not chewed, crushed, or broken prior to swallowing.

Dosing Scheme for Ramp-Up Phase

Day	Venetoclax Daily Dose
1	100 mg
2	200 mg
3	400 mg
4 and beyond	400 mg

The Starting Pack provides the first 4 days of Venetoclax according to the ramp-up schedule.

Patients treated with Venetoclax may develop tumor lysis syndrome. Refer to the appropriate section below for specific details on management. Assess patient-specific factors for level of risk of tumor lysis syndrome (TLS) and provide prophylactic hydration and anti-hyperuricemics to patients prior to first dose of Venetoclax to reduce risk of TLS as clinically indicated.

Acute Myeloid Leukemia

- All patients should have white blood cell count less than 25×10^9/L prior to initiation of Venetoclax. Cytoreduction prior to treatment may be required.
- Prior to first Venetoclax dose, provide all patients with prophylactic measures including adequate hydration and anti-hyperuricemic agents and continue during ramp-up phase as clinically indicated.
- Assess blood chemistry (potassium, uric acid, phosphorus, calcium, and creatinine) and correct pre-existing abnormalities prior to initiation of treatment with Venetoclax.
- Monitor blood chemistries for TLS at pre-dose, daily with each new dose during ramp-up and 24 hours after reaching final dose.
- For patients with risk factors for TLS (e.g., circulating blasts, high burden of leukemia involvement in bone marrow, elevated pretreatment lactate dehydrogenase (LDH) levels, or reduced renal function) additional measures should be considered, including hospitalization, hydration, rasburicase, more frequent monitoring of TLS labs, and other measures as deemed necessary by treating physician.

6.1.2. Maintenance therapy phase

The first 6 cycles of azacitidine and venetoclax constitute induction phase for purposes of this protocol. After induction phase is completed, it is planned that an additional 2 years, up to a maximum of 36 doses of pembrolizumab will be administered for patients in response from this regimen during the maintenance phase. For patients on the control arm, treatment with azacitidine and venetoclax may continue for 2 years after the 6 cycles (up to additional 24 cycles) during the maintenance phase. No dose ramp-up is required during the maintenance therapy phase. Patients whose best response to therapy after 6 cycles is stable disease can only remain on study therapy if they derive clinical benefit from therapy that is defined by transfusion independence (defined as at least 8 consecutive weeks without transfusions of either red blood cells or platelets for patients who were receiving transfusions prior to or during therapy) or by hematologic improvement criteria listed in section 12.2.5. of the protocol.

Continuation of azacitidine and venetoclax for patients completing 24 cycles of therapy may be continued for patients in response or with stable disease at discretion of treating investigator. Patients who do not remain in clinical response will be taken off treatment/study. Patients in response will proceed with AZA + VEN with pembrolizumab (intervention arm) *vs*. AZA + VEN (control arm) every Q3W dosing schedule for up to 2 years. Bone marrow samples will be obtained every 3 months while patients are receiving maintenance to assess for MRD and perform correlative studies (optional during maintenance). Patients, who are deemed to require HSCT, will forgo any remaining protocol-defined maintenance therapy and proceed with HSCT. Patients proceeding with HSCT will be followed for survival and SAEs through the first 100 days post-HSCT including assessment for occurrence of acute graft versus host disease (GVHD).

Maintenance Regimen Description					
Agent	**Premedications ; Precautions**	**Dose***	**Route#**	**Schedule**	**Cycle Length**
Pembrolizumab (for intervention arm only)	Premedication with antipyretic and antihistamine may be considered. No premedication with steroids	200 mg See Section 8.1.1 for instructions on the preparation of the infusion solution.	IV over 30 minutes (between 25-40 minutes) using an infusion set containing a low-protein binding 0.2 to 5 µm in-line filter. See Section 8.1 for compatible infusion set materials including in-line filter.	Q3W ±3 days	28 days
Azacitidine	IV formulation is incompatible with 5% dextrose, Hespan, or bicarbonate solutions. Patients should be pre-medicated for nausea and vomiting.	75 mg/m^2 Refer to Section 8.2.1 for additional information.	IV over 10-40 minutes or SQ	Days 1-7 or days 5-2-2 (to avoid weekend administration)	

Maintenance Regimen Description					
Agent	**Premedications ; Precautions**	**Dose***	**Route#**	**Schedule**	**Cycle Length**
Venetoclax	None.	400 mg (refer to ramp up dosing scheme) Refer to Section 8.2.2 for additional informatio n.	P.O.	Days 1-21 or 1-28 dosing (depending count recovery) for all cycles.	
*Dose rounding per institutional guidelines is allowed. #Order should be pembrolizumab first, followed by azacitidine at least an hour later on days when both medications are given.					

6.1.3. CTEP IND Agent

Trial treatment of Pembrolizumab will be administered Day 8 of the induction phase of the study. Trial treatment may be administered up to 3 days before or after the scheduled day due to administrative reasons.

Note: Dosing interruptions are permitted in the case of medical/surgical events or logistical reasons (*i.e*., elective surgery, unrelated medical events, patient vacation, holidays) not related to study therapy. Patients should be placed back on study therapy within 3 weeks of the scheduled interruption. . If interruption is longer than 3 weeks, patient can resume study therapy with study chair approval. The reason for interruption should be documented in the patient's study record.

Pembrolizumab (MK-3475) will be administered as a dose of 200 mg using a 30-minute IV infusion. Infusion timing should be as close to 30 minutes as possible; however, a window of -5 minutes and +10 minutes is permitted (*i.e*., infusion time is 25-40 minutes

6.2. General Concomitant Medication and Supportive Care Guidelines

The study team should check a frequently-updated medical reference for a list of drugs to avoid or minimize use of. Appendix C (Patient Clinical Trial Wallet Card) should be provided to patients.

6.2.1. Pembrolizumab Concomitant Medication

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for any medication or vaccination specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be

required. The investigator should discuss any questions regarding this with CTEP. The final decision on any supportive therapy or vaccination rests with the investigator and/or the patient's primary physician; however, the decision to continue the patient on trial therapy or vaccination schedule requires the mutual agreement of the Investigator, CTEP, and the patient.

Acceptable Concomitant Medications
All treatments that the investigator considers necessary for a patient's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medication will be recorded on the case report form (CRF) including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date may also be included on the CRF.

All concomitant medications received within 30 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered after 30 days after the last dose of trial treatment should be recorded for serious AEs (SAEs).

Prohibited Concomitant Medications
Patients are prohibited from receiving the following therapies during the Screening and Treatment Phase (including retreatment for post-complete response relapse) of this trial:

- Anti-leukemia Immunotherapy not specified in this protocol.
- Anti-leukemia Chemotherapy not specified in this protocol. Note: If patient needs intrathecal chemotherapy after start of trial therapy on study as prophylaxis or for new development of CNS leukemia, patient is allowed to continue on study.
- Anti-leukemia investigational agents other than pembrolizumab (MK-3475).
- Radiation therapy
  - Note: Radiation therapy to a symptomatic solitary lesion or to the brain may be considered on an exceptional case by case basis after consultation with CTEP. The patient must have clear measurable disease outside the radiated field. Administration of palliative radiation therapy will be considered clinical progression for the purposes of determining PFS.
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, chicken pox, yellow fever, rabies, Bacillus Calmette–Guérin (BCG), and typhoid (oral) vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (*e.g.*, Flu-Mist®) are live attenuated vaccines, and are not allowed.
- Once on and during the treatment phase of the trial, high dose systemic steroid therapy should be avoided as much as possible but is not strictly prohibited.

Patients who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the trial except if they are allowed

to continue on study on case-by-case basis based on consultation with study chair and CTEP. Patients may receive other medications that the investigator deems to be medically necessary.

The Exclusion Criteria describe other medications which are prohibited in this trial.
There are no prohibited therapies during the Post-Treatment Follow-up Phase.

6.2.2. Pembrolizumab Supportive Care Guidelines

Patients should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of AEs with potential immunologic etiology are also outlined in the table in Section 7.1.3. Where appropriate, these guidelines include the use of oral or IV treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the investigator determines the events to be related to Pembrolizumab.

Note: If after the evaluation the event is determined not to be related, the investigator does not need to follow the treatment guidance (as outlined below).

It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of the evaluation of the event.

6.2.3. Venetoclax Concomitant Medications

Venetoclax is predominantly metabolized by CYP3A4 *in vitro*, thus CYP3A4 inhibitors or inducers are expected to cause changes in venetoclax exposures (Venetoclax Investigator's Brochure, 2018). Clinical studies have supported the *in vitro* observations for venetoclax as a sensitive substrate of CYP3A4: >5-fold increase in AUC when co-dosed with ketoconazole, and 71% decrease in AUC when co-dosed with rifampin. Venetoclax is a substrate for the efflux transporters P-gp and BCRP, and inhibitors or inducers of these transporters are expected to cause change in the exposure of venetoclax. Venetoclax is a P-gp and BCRP inhibitor and may interact with substrates for these transporters. Venetoclax may inhibit OATP1B1 and cause weak interaction with drugs that are substrates of this transporter. Refer to the venetoclax label for dose adjustment concerning concurrent medications.

6.2.3.1. Excluded Concomitant Medications and Procedures
CYP3A inhibitors and inducers are allowed on study as clinically indicated and per standard institutional uses but their use including dose adjustment of venetoclax and other drugs should follow the FDA drug label for venetoclax

6.3. Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment may continue for up to 24 cycles or 2 years of maintenance therapy or until one of the following criteria applies:

- Disease progression
- Once patient proceeds to HSCT
- Intercurrent illness that prevents further administration of treatment
- AE(s) which require(s) treatment discontinuation (see also Section 7):
  - Any dosing interruption lasting >12 weeks with the following exceptions: Dosing interruptions >12 weeks that occur for non-drug-related reasons may be allowed if approved by the Principal Investigator. Prior to re-initiating treatment in a patient with a dosing interruption lasting >12 weeks, the Principal Investigator must be consulted.
  - Tumor assessments should continue as per protocol even if dosing is interrupted.
- Unacceptable AE(s)
- Patient decides to withdraw from the study
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Clinical progression
- Severe, significant and persistent Patient non-compliance
- Pregnancy
  - All women of child bearing potential should be instructed to contact the investigator immediately if they suspect they might be pregnant (*e.g.*, missed or late menstrual period) at any time during study participation.
  - The investigator must immediately notify CTEP in the event of a confirmed pregnancy in a patient participating in the study.
- Termination of the study by sponsor
- The drug manufacturer can no longer provide the study agent

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

Patients with grade 3-4 IRAEs that completely resolve maybe allowed to continue on study on case-by-case basis based on consultation with study chair and CTEP

6.4. Duration of Follow-Up

Patients will be followed in clinic or phone call for at least every 6 months for up to 3 years after treatment or until death, whichever occurs first. Patients removed from study for unacceptable AE(s) will be followed until resolution or stabilization of the AE.

6.5. Criteria to Resume Treatment

For non-autoimmune or inflammatory events, patients may resume treatment with study drug when the drug-related AE(s) resolve to ≤ grade 1 or baseline value, with the following exceptions:

- Patients may resume treatment in the presence of grade 2 fatigue.
- Patients with baseline grade 1 AST/ALT or total bilirubin who require dose delays for reasons other than a 2-grade shift in AST/ALT or total bilirubin may resume treatment in the presence of grade 2 AST/ALT OR total bilirubin.
- Patients with combined grade 2 AST/ALT and total bilirubin values meeting study parameters outlined in Section 7.1 which are attributed to study therapy should have treatment permanently discontinued.
- Non-drug-related toxicity including hepatic, pulmonary toxicity, diarrhea, or colitis, must have resolved to baseline before treatment is resumed.
- Drug-related endocrinopathies (not including drug-related adrenal insufficiency or hypophysitis) adequately controlled with only physiologic hormone replacement may resume treatment after replacement correction and clinically stable regimen.

If the criteria to resume treatment are met, the patient should restart treatment no sooner than the next scheduled time point per protocol. However, if the treatment is delayed past the next scheduled time point per protocol, the treatment should resume at the earliest convenient point that is within the 12-week delay period.

If treatment is delayed >12 weeks, the patient must be permanently discontinued from study therapy, except as specified in Section 6.3 (Duration of Therapy) or except if they are allowed to continue on study on case-by-case basis based on consultation with study chair and CTEP.

Patients with grade 3-4 IRAEs that completely resolve may be allowed to continue on study on case-by-case basis based on consultation with study chair and CTEP

6.6. Contraception and Pregnancy

6.6.1 Contraception

Pembrolizumab may have adverse effects on a fetus in utero. Furthermore, it is not known if Pembrolizumab has transient adverse effects on the composition of sperm.

For this trial, male patients will be considered to be of nonreproductive potential if they have azoospermia (whether due to having had a vasectomy or due to an underlying medical condition).

Female patients will be considered of nonreproductive potential if they are either:

- Postmenopausal (defined as at least 12 months with no menses without an alternative medical cause; in women <45 years of age, a high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy. In the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.);
  OR

- Have had a hysterectomy and/or bilateral oophorectomy, bilateral salpingectomy or bilateral tubal ligation/occlusion, at least 6 weeks prior to screening;
  OR
- Has a congenital or acquired condition that prevents childbearing.

Female and male patients with female partners of reproductive potential must agree to avoid becoming pregnant or impregnating a partner, respectively, while receiving study drug and for 120 days after the last dose of study drug by complying with one of the following:

- Practice abstinence[†] from heterosexual activity;
  OR
- Use (or have their partner use) acceptable contraception during heterosexual activity.

Acceptable methods of contraception are[‡]:

Single method (1 of the following is acceptable):
- intrauterine device (IUD)
- vasectomy of a female patient's male partner
- contraceptive rod implanted into the skin

Combination method (requires use of 2 of the following):
- diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)
- cervical cap with spermicide (nulliparous women only)
- contraceptive sponge (nulliparous women only)
- male condom or female condom (cannot be used together)
- hormonal contraceptive: oral contraceptive pill (estrogen/progestin pill or progestin-only pill), contraceptive skin patch, vaginal contraceptive ring, or subcutaneous contraceptive injection

[†]Abstinence (relative to heterosexual activity) can be used as the sole method of contraception if it is consistently employed as the patient's preferred and usual lifestyle and if considered acceptable by local regulatory agencies and Ethics Review Committees (ERCs)/Institutional Review Boards (IRBs). Periodic abstinence (*e.g.*, calendar, ovulation, sympto-thermal, post-ovulation methods, etc.) and withdrawal are not acceptable methods of contraception.

[‡]If a contraceptive method listed above is restricted by local regulations/guidelines, then it does not qualify as an acceptable method of contraception for patients participating at sites in this country/region.

Patients should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study, patients of childbearing potential must adhere to the contraception requirement (described above) from the day of study medication initiation (or 14 days prior to the initiation of study medication for oral contraception) throughout the study period up to 120 days after the last dose

of trial therapy. If there is any question that a patient will not reliably comply with the requirements for contraception, that patient should not be entered into the study.

6.6.2 Use in Pregnancy

If a patient inadvertently becomes pregnant while on treatment with Pembrolizumab, the patient will immediately be removed from the study. The site will contact the patient at least monthly and document the patient's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported without delay and within 24 hours if the outcome is a serious adverse experience (*e.g.*, death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn). The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn. If a male patient impregnates his female partner, the study personnel at the site must be informed immediately and the pregnancy reported and followed.

6.6.3 Use in Nursing Women

It is unknown whether Pembrolizumab is excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, patients who are breast-feeding are not eligible for enrollment.

6.7 Treatment Up to 2 Years

Maintenance treatment will continue for up to 2 years (up to 24 cycles from start of maintenance, and for those randomized to intervention arm, maximum of 36 doses of Pembrolizumab (MK-3475), documented disease progression, unacceptable AE(s), intercurrent illness that prevents further administration of treatment, investigator's decision to withdraw the patient, patient proceeding to HSCT, patient withdraws consent, pregnancy of the patient, noncompliance with trial treatment or procedure requirements, or administrative reasons.

7. DOSING DELAYS/DOSE MODIFICATIONS

Dosing delays and dose modifications for therapy should follow institutional guidelines and/or treating physicians opinion.

7.1 Pembrolizumab (MK-3475)

7.1.1 Immune-related AEs

Immune-related AEs (irAEs), defined as AEs of unknown etiology, associated with drug exposure and consistent with an immune phenomenon, may be predicted based on the nature of the pembrolizumab compound, its mechanism of action, and reported experience with immunotherapies that have a similar mechanism of action. Special attention should be paid to AEs that may be suggestive of potential irAEs. An irAE can occur shortly after the first dose or several months after the last dose of treatment. All AEs of unknown etiology associated with drug exposure should be evaluated to determine if they are possibly immune-related. If an irAE

is suspected, efforts should be made to rule out neoplastic, infectious, metabolic, toxin or other etiologic causes prior to labeling an AE as an irAE.

The table below includes guidelines for managing irAEs.

General Dose Modification Guidelines for Drug-Related Immune-Related Adverse Events

irAE	Withhold/Discontinue pembrolizumab (MK-3475)?	Supportive Care
Grade 1	No action.	Provide symptomatic treatment.
Grade 2	May withhold pembrolizumab	Consider systemic corticosteroids in addition to appropriate symptomatic treatment.
Grade 3 and Grade 4	Withhold pembrolizumab Discontinue if unable to reduce corticosteroid dose to <10 mg per day prednisone equivalent within 12 weeks of toxicity.	Systemic corticosteroids are indicated in addition to appropriate symptomatic treatment. May utilize 1 to 2 mg/kg prednisone or equivalent per day. Steroid taper should be considered once symptoms improve to grade 1 or less and tapered over at least 4 weeks.

7.1.2 Non-immune-related Adverse Events

Additionally, pembrolizumab (MK-3475) will be withheld for other drug-related grade 4 hematologic toxicities, drug-related non-hematological toxicity ≥ grade 3 including laboratory abnormalities, and severe or life-threatening AEs. The table below includes dose modification guidelines for other toxicities that do not appear to be irAEs. Note: For hematological AEs, these guidelines are intended to be applied when the investigator determines the events to be Pembrolizumab-related. Dose modifications of MK- 3475 (pembrolizumab) for hematological AEs do not apply during the induction phase of treatment. Note: if after the evaluation the event is determined not to be related, the investigator does not need to follow the treatment guidance. Therefore, these recommendations should be seen as guidelines and the treating physician should exercise individual clinical judgment based on the patient.

Dose Modification Guidelines for Non-IRAE-Related Adverse Events

Toxicity	Grade	Hold Treatment (Y/N)	Timing for restarting treatment	Dose/Schedule for restarting treatment	Discontinue Subject
Hematological Toxicity*	1, 2, 3	No	N/A	N/A	N/A
	4	Yes	Toxicity resolves to grade 0-1 or baseline	May increase the dosing interval by 1 week	Toxicity does not resolve within 12 weeks of last infusion. *Permanent discontinuation should be considered for any severe or life-threatening event.*
Non-hematological toxicity **NOTE:** Exception to be treated similar to grade 1 toxicity • Grade 2 alopecia • Grade 2 fatigue For additional information regarding Adverse Events with a potential Immune-Etiology reference Section 7.1.1.	1	No	N/A	N/A	N/A
	2	Consider withholding for persistent symptoms	Toxicity resolves to grade 0-1 or baseline	*Clinical AE resolves within 4 weeks*: Same dose and schedule *Clinical AE does not resolve within 4 weeks:* May increase the dosing interval by 1 week for each occurrence	Toxicity does not resolve within 12 weeks of last infusion
	3	Yes	Toxicity resolves to grade 0-1 or baseline	May increase the dosing interval by 1 week for each occurrence	Toxicity does not resolve within 12 weeks of last infusion
	4	Yes	N/A	N/A	Subject must be discontinued
*Only if deemed related to pembrolizumab (MK-3475) treatment					

In case toxicity does not resolve to grade 0-1 within 12 weeks after last infusion, protocol treatment should be discontinued. With Study Chair agreement, subjects with a laboratory AE still at grade 2 after 12 weeks may continue treatment in the trial only if asymptomatic and controlled. Patients who experience a recurrence of the same severe or life-threatening event at the same grade or greater with re-challenge of pembrolizumab (MK-3475) should be discontinued from protocol treatment.

7.1.3 Pembrolizumab Dose Modifications and Supportive Care Guidelines for Drug-Related Adverse Events

7.1.4 Dose Modifications

Adverse events (both nonserious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These AEs may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as the table in Section ***

Dosing interruptions are permitted in the case of medical/surgical events or logistical reasons not related to study therapy (*e.g.*, elective surgery, unrelated medical events, patient vacation, and/or holidays). Patients should be placed back on study therapy within 3 weeks of the scheduled interruption. The reason for interruption should be documented in the patient's study record.

7.1.5. Supportive Care Guidelines

Patients should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of AEs with potential immunologic etiology are also outlined in the table in Section 1.1.3. Where appropriate, these guidelines include the use of oral or IV treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the investigator determines the events to be related to pembrolizumab.

Note: If after the evaluation the event is determined not to be related, the investigator does not need to follow the treatment guidance (as outlined below).

It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of the evaluation of the event.

7.1.6 Dose Modification and Toxicity Management for Immune-related Adverse Events Associated with Pembrolizumab

AEs associated with pembrolizumab exposure may represent an immunologic etiology. These immune-related AEs (irAEs) may occur shortly after the first dose or several months after the last dose of pembrolizumab treatment and may affect more than one body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. Based on existing clinical study data, most irAEs were reversible and could be managed with interruptions of pembrolizumab, administration of corticosteroids and/or other supportive care. For suspected irAEs, ensure adequate evaluation to confirm etiology or exclude other causes. Additional procedures or tests such as bronchoscopy, endoscopy, skin biopsy may be included as part of the evaluation. Based on the severity of irAEs, withhold or permanently discontinue pembrolizumab and administer corticosteroids. Pembrolizumab may cause severe or life-threatening infusion-reactions including severe hypersensitivity or anaphylaxis. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Dose modification and toxicity management guidelines for irAEs and infusion reactions associated with pembrolizumab are provided in the table below.

Note that non-irAEs will be managed as appropriate, following clinical practice recommendations.

Table Dose Modification and Toxicity Management Guidelines for Immune-related AEs and Infusion Reactions Associated with Pembrolizumab

General instructions:

1. For non-endocrine-related severe and life-threatening irAEs, investigators should consider the use of IV corticosteroids followed by oral steroids. Other immunosuppressive treatment should begin if the irAEs are not controlled by corticosteroids. Some non-endocrine irAEs do not require steroids. For example, celiac disease induced by pembrolizumab can be controlled by diet alone.
2. For non-endocrine-related toxicities, pembrolizumab must be permanently discontinued if the irAE does not resolve or the corticosteroid dose is not ≤10 mg/day within 12 weeks of the last pembrolizumab-treatment.
3. Generally, when corticosteroids are used, investigators should begin a taper when the irAE is ≤Grade 1 and continue at least 4 weeks.
4. If pembrolizumab has been withheld due to a non-endocrine irAE, pembrolizumab may generally resume after the irAE has decreased to ≤Grade 1 after a corticosteroid taper.

irAEs	**Toxicity grade (CTCAE V5.0)**	**Action with pembrolizumab**	**Corticosteroid and/or other therapies**	**Monitoring and follow-up**
Pneumonitis	Grade 2	Withhold	Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper Add prophylactic antibiotics for opportunistic infections	Monitor participants for signs and symptoms of pneumonitis Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment
	Recurrent Grade 2, Grade 3 or 4	Permanently discontinue		
Diarrhea / Colitis	Grade 2 or 3	Withhold	Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper Patients who do not respond to corticosteroids should be seen by a gastroenterologist for confirmation of the diagnosis and consideration of secondary immune suppression	Monitor participants for signs and symptoms of enterocolitis (*i.e.*, diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (*i.e.* peritoneal signs and ileus) Specifically assess for celiac disease serologically, and exclude *Clostridium difficile* infection Participants with ≥Grade 2 diarrhea suspecting enterocolitis should consider GI consultation and performing endoscopy to rule out enterocolitis and assess mucosal severity Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion
	Recurrent Grade 3 or Grade 4	Permanently discontinue		

AST or ALT elevation or Increased Bilirubin	Grade 2 [a]	Withhold	Administer corticosteroids (initial dose of 0.5 to 1 mg/kg prednisone or equivalent) followed by taper	Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable)
	Grade 3 [b] or 4 [c]	Permanently discontinue	Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper	
Type 1 diabetes mellitus (T1DM) or Hyperglycemia	Grade 1 or 2	Continue		Investigate for diabetes. In the absence of corticosteroids or diabetes medication non-adherence, any grade hyperglycemia may be an indication of beta-cell destruction and pembrolizumab-induced diabetes akin to type 1 diabetes. This should be treated as a Grade 3 event. Given this risk, exercise caution in utilizing non-insulin hypoglycemic agents in this setting. After a thorough investigation of other potential causes, which may involve a referral to an endocrinologist, follow institutional guidelines. Monitor glucose control.
	New onset T1DM (evidence of β-cell failure) or Grade 3 or 4 hyperglycemia	Withhold [d] Resume pembrolizumab when symptoms resolve and glucose levels are stable	Initiate treatment with insulin If patient is found to have diabetic ketoacidosis or hyperglycemic hyperosmolar syndrome, treat as per institutional guidelines with appropriate management and laboratory values (*e.g.* anion gap, ketones, blood pH, *etc.*) reported	Monitor for glucose control Strongly consider referral to endocrinologist Obtain C-peptide level paired with glucose, autoantibody levels (*e.g.* GAD65, islet cell autoantibodies), and hemoglobin A1C level
Hypophysitis	Grade 2	Withhold	Administer corticosteroids and initiate hormonal	Monitor for signs and symptoms of

	Grade 3 or 4	Withhold or permanently discontinue [d]	replacements as clinically indicated	hypophysitis (including hypopituitarism and adrenal insufficiency) Provide adrenal insufficiency precautions including indications for stress dose steroids and medical alert jewelry Strongly consider referral to endocrinologist
Hyperthyroidism	Grade 2	Consider withholding. Resume pembrolizumab when symptoms are controlled, and thyroid function is improving	Treat with nonselective beta-blockers (*e.g.*, propranolol) or thionamides as appropriate Initiate treatment with anti-thyroid drug such as methimazole or carbimazole as needed	Monitor for signs and symptoms of thyroid disorders Strongly consider referral to endocrinologist
	Grade 3 or 4	Withhold or permanently discontinue [d]		
Hypothyroidism	Grade 2, 3 or 4	Continue	Initiate thyroid replacement hormones (*e.g.*, levothyroxine or liothyronine) per standard of care	Monitor for signs and symptoms of thyroid disorders
Nephritis: grading according to increased creatinine or acute kidney injury	Grade 2	Withhold	Administer corticosteroids (prednisone 1 to 2 mg/kg or equivalent) followed by taper	Monitor changes of renal function Strongly consider referral to nephrologist
	Grade 3 or 4	Permanently discontinue		
Cardiac Events (including myocarditis, pericarditis, arrhythmias, impaired ventricular function, vasculitis)	Asymptomatic cardiac enzyme elevation with clinical suspicion of myocarditis (previously CTCAE v4.0 Grade 1), or Grade 1	Withhold	Based on severity of AE administer corticosteroids	Ensure adequate evaluation to confirm etiology and/or exclude other causes Strongly consider referral to cardiologist and cardiac MRI Consider endomyocardial biopsy If event resolves to Grade 1 or better, taper corticosteroids over ≥1 month

	Grade 2, 3 or 4	Permanently discontinue	Initiate treatment with corticosteroids equivalent to 1-2 mg/kg/day IV methylprednisolone and convert to 1-2 mg/kg/day oral prednisone or equivalent upon improvement If event does not improve within 48 hours after initiating corticosteroids, consider adding an immunosuppressive agent Initiate treatment per institutional guidelines and consider antiarrhythmic drugs, temporary pacemaker, extracorporeal membrane oxygenation (ECMO), ventricular assist device (VAD), or pericardiocentesis as appropriate	Ensure adequate evaluation to confirm etiology and/or exclude other causes Strongly consider referral to cardiologist and cardiac MRI Consider endomyocardial biopsy If event resolves to Grade 1 or better, taper corticosteroids over ≥1 month
Exfoliative Dermatologic Conditions	Suspected SJS, TEN, or DRESS	Withhold	Based on severity of AE administer corticosteroids	Ensure adequate evaluation to confirm etiology or exclude other causes Strongly consider referral to dermatologist Consider skin biopsy for evaluation of etiology
	Confirmed SJS, TEN, or DRESS	Permanently discontinue		
All Other irAEs	Persistent Grade 2	Withhold	Based on severity of AE administer corticosteroids	Ensure adequate evaluation to confirm etiology or exclude other causes
	Grade 3	Withhold or discontinue based on the event [e]		
	Recurrent Grade 3 or Grade 4	Permanently discontinue		

Infusion-Related Reactions

Infusion Reactions	NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Mild reaction; infusion interruption not indicated; intervention not indicated	Grade 1	Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator.	None
Requires therapy or infusion interruption but responds promptly to symptomatic treatment (*e.g.*, antihistamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for ≤24 hrs.	Grade 2	• **Stop Infusion.** • Additional appropriate medical therapy may include but is not limited to: • IV fluids • Antihistamines • NSAIDs • Acetaminophen • Narcotics • Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. • If symptoms resolve within 1 hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (*e.g.* from 100 mL/hr. to 50 mL/hr.). Otherwise dosing will be held until symptoms resolve and the participant should be premedicated for the next scheduled dose. **Participants who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further study drug treatment**	Participant may be premedicated 1.5h (± 30 minutes) prior to infusion of study intervention with: Diphenhydramine 50 mg PO (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg PO (or equivalent dose of analgesic).

Infusion Reactions	NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Prolonged (*i.e.*, not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates)	Grade 3	• **Stop Infusion.** • Additional appropriate medical therapy may include but is not limited to: • Epinephrine** • IV fluids • Antihistamines • NSAIDs • Acetaminophen • Narcotics • Oxygen • Pressors • Corticosteroids (*e.g.* methylprednisolone 2 mg/kg/day or dexamethasone 10 mg every 6 hours) • Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. • Hospitalization may be indicated. **In cases of anaphylaxis, epinephrine should be used immediately. Participant is permanently discontinued from further study drug treatment.	No subsequent dosing.
Life-threatening; pressor or ventilator support indicated	Grade 4	Admit participant to intensive care unit (ICU) and initiate hemodynamic monitoring, mechanical ventilation, and/or IV fluids and vasopressors as needed. Monitor other organ function closely. Manage constitutional symptoms and organ toxicities as per institutional practice. Follow Grade 3 recommendations as applicable.	No subsequent dosing.

Infusion Reactions	NCI CTCAE Grade	Treatment	Premedication at subsequent dosing

AE(s)=adverse event(s); ALT= alanine aminotransferase; AST=aspartate aminotransferase; CTCAE=Common Terminology Criteria for Adverse Events; DRESS=Drug Rash with Eosinophilia and Systemic Symptom; ECMO=extracorporeal membrane oxygenation; GI=gastrointestinal; ICU=intensive care unit; IO=immuno-oncology; ir=immune related; IV=intravenous; MRI=magnetic resonance imaging; PO=per os; SJS=Stevens-Johnson Syndrome; T1DM=type 1 diabetes mellitus; TEN=Toxic Epidermal Necrolysis; ULN=upper limit of normal; VAD=ventricular assist device.

Note: Non-irAE will be managed as appropriate, following clinical practice recommendations.

[a] AST/ALT: >3.0 to 5.0 x ULN if baseline normal; >3.0 to 5.0 x baseline, if baseline abnormal; bilirubin:>1.5 to 3.0 x ULN if baseline normal; >1.5 to 3.0 x baseline if baseline abnormal

[b] AST/ALT: >5.0 to 20.0 x ULN, if baseline normal; >5.0 to 20.0 x baseline, if baseline abnormal; bilirubin:>3.0 to 10.0 x ULN if baseline normal; >3.0 to 10.0 x baseline if baseline abnormal

[c] AST/ALT: >20.0 x ULN, if baseline normal; >20.0 x baseline, if baseline abnormal; bilirubin: >10.0 x ULN if baseline normal; >10.0 x baseline if baseline abnormal

[d] The decision to withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician. If control achieved or ≤Grade 2, pembrolizumab may be resumed.

[e] Events that require discontinuation include but are not limited to: encephalitis and other clinically important irAEs (*e.g*. vasculitis and sclerosing cholangitis).

Appropriate resuscitation equipment should be available at the bedside and a physician readily available during the period of drug administration. For further information, please refer to the Common Terminology Criteria for Adverse Events v5.0 (CTCAE) at http://ctep.cancer.gov.

Neurological Toxicities

Event	Management
Immune-mediated neuropathy, Grade 1	• Continue pembrolizumab. • Investigate etiology. • Any cranial nerve disorder (including facial paresis) should be managed as per Grade 2 management guidelines below.

Immune-mediated neuropathy, including facial paresis, Grade 2	• Withhold pembrolizumab for up to 12 weeks after event onset. [a]
	• Investigate etiology and refer patient to neurologist.
	• Initiate treatment as per institutional guidelines.
	• For general immune-mediated neuropathy:
	  o If event resolves to Grade 1 or better, resume pembrolizumab. [b]
	  o If event does not resolve to Grade 1 or better while withholding pembrolizumab, permanently discontinue pembrolizumab. [c]
	• For facial paresis:
	  o If event resolves fully, resume pembrolizumab. [b]
	  o If event does not resolve fully while withholding pembrolizumab, permanently discontinue pembrolizumab. [c]

Immune-mediated neuropathy, including facial paresis, Grade 3 or 4	• Permanently discontinue pembrolizumab. [c] • Refer patient to neurologist. • Initiate treatment as per institutional guidelines.
Myasthenia gravis and Guillain-Barré syndrome (any grade)	• Permanently discontinue pembrolizumab. [c] • Refer patient to neurologist. • Initiate treatment as per institutional guidelines. • Consider initiation of corticosteroids equivalent to 1–2 mg/kg/day oral or IV prednisone.

[a] Pembrolizumab may be withheld for a longer period of time (*i.e*., >12 weeks after event onset) to allow for

corticosteroids (if initiated) to be reduced to the equivalent of ≤10 mg/day oral prednisone. The acceptable length of the extended period of time must be based on an assessment of benefit–risk by the investigator and in alignment with the protocol requirements for the duration of treatment and documented by the investigator.
[b] If corticosteroids have been initiated, they must be tapered over ≥1 month to the equivalent of ≤10 mg/day oral prednisone before pembrolizumab can be resumed.
[c] Resumption of pembrolizumab may be considered in patients who are deriving benefit and have fully recovered from the immune-mediated event. The decision to re-challenge patients with pembrolizumab should be based on investigator's assessment of benefit–risk and documented by the investigator (or an appropriate delegate).

7.2 Venetoclax

Monitor blood counts frequently through resolution of cytopenias. Management of some adverse reactions may require dose interruptions or permanent discontinuation of VENCLEXTA. shows the dose modification guidelines for hematologic toxicities. Below are guidelines and ultimate decision on dose modifications of venetoclax is per the treating physician's judgement.

Event	Occurrence	Action
Hematological Toxicity		
Grade 4 neutropenia with or without fever or infection; or Grade 4 thrombocytopenia	Occurrence prior to achieving remission	Transfuse blood products, administer prophylactic and treatment anti-infectives as clinically indicated. In most instances, VENCLEXTA and azacitidine cycles should not be interrupted due to cytopenias prior to achieving remission
	First occurrence achieving remission and lasting at least 7 days	Delay subsequent treatment cycle of VENCLEXTA and azacitidine and monitor blood counts. Administer granulocyte-colony stimulating factor (G-CSF) if clinically indicated for neutropenia. Once the toxicity has resolved to Grade 1 or 2, resume VENCLEXTA therapy at the same dose in combination with azacitidine.

	Subsequent occurrences in cycles after achieving remission and lasting 7 days or longer	Delay subsequent treatment cycle of VENCLEXTA and azacitidine, and monitor blood counts. Administer G-CSF if clinically indicated for neutropenia. Once the toxicity has resolved to Grade 1 or 2, resume VENCLEXTA therapy at the same dose and the duration reduced.
Adverse reactions were graded using NCI CTCAE version 4.0.		

7.3 Azacitidine

Below are guidelines for azacitidine dose modification that are per the drug label. However, the ultimate decision on dose modifications of azacitidine is per the treating physician's judgement. Generally, dose reductions of azacitidine for hematological values are not recommended before achievement of morphologic remission.

For patients with baseline (start of treatment) white blood count (WBC) ≥3 x10^9/L, ANC ≥1.5 x10^9/L, and platelets ≥75 x10^9/L, adjust the dose as follows, based on nadir counts for any given cycle:

Nadir counts		**Azacitidine dose in the next course (mg/m^2)**
ANC (x10^9/L)	**Platelets (x10^9/L)**	
<0.5	<25	37.5
0.5-1.5	25-50	50
>1.5	>50	75
ANC=absolute neutrophil count		

For patients whose baseline counts are WBC <3 x10^9/L, ANC <1.5 x10^9/L, or platelets <75 x10^9/L, dose adjustments should be based on nadir counts and bone marrow biopsy cellularity at the time of the nadir as noted below, unless there is clear improvement in differentiation (percentage of mature granulocytes is higher and ANC is higher than at onset of that course) at the time of the next cycle, in which case the dose of the current treatment should be continued.

WBC or platelet nadir % decrease in counts from baseline	**Bone marrow biopsy cellularity % At time of nadir**		
	30-60	15-30	<15
	Azacitidine dose in the Next Course (mg/m^2)		
50-75	75	37.5	25
>75	56.5	37.5	25
WBC=white blood count			

If a nadir as defined in the table above has occurred, the next course of treatment should be given 28 days after the start of the preceding course, provided that both the WBC and the platelet counts are >25% above the nadir and rising. If a >25% increase above the nadir is not seen by Day 28, counts should be reassessed every 7 days. If a 25% increase is not seen by Day 42, then the patient should be treated with 50% of the scheduled dose (37.5 mg/m^2).

If unexplained reductions in serum bicarbonate levels to less than 20 mEq/L occur, the dosage should be reduced by 50% (to 37.5 mg/m^2) on the next course. Similarly, if unexplained elevations of blood urea nitrogen (BUN) or serum creatinine occur, the next cycle should be delayed until values return to normal or baseline and the dose should be reduced by 50% (to 37.5 mg/m^2) on the next treatment course. (See Vidaza® Package Insert)

Azacitidine and its metabolites are known to be substantially excreted by the kidney, and the risk of toxic reactions to this drug may be greater in patients with impaired renal function. Because

elderly patients are more likely to have decreased renal function, care should be taken in dose selection, and it may be useful to monitor renal function. (See Vidaza® Package Insert)

8 PHARMACEUTICAL AGENT INFORMATION

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

8.1 CTEP IND Agent

8.1.1 Pembrolizumab (MK-3475) NSC # 776864

Other Names: Pembrolizumab, SCH 900475, KEYTRUDA®

Classification: Anti-PD-1 MAb

Molecular Weight: 148.9-149.5 KDa

CAS Number: 1374853-91-4

Mode of Action: The programmed cell death 1 (PD-1) receptor is an inhibitory receptor expressed by T-cells. When bound to either of its ligands, PD-L1 or PD-L2, activated PD-1 negatively regulates T-cell activation and effector function. The pathway may be engaged by tumor cells to suppress immune control. Pembrolizumab (MK-3475) blocks the negative immune regulatory signaling by binding to the PD-1 receptor, inhibiting the interaction between PD-1 and its ligands.

Description: Pembrolizumab (MK-3475) is a humanized MAb of the IgG4/kappa isotype.

How Supplied: Pembrolizumab (MK-3475) is supplied by Merck & Co., Inc. and distributed by the Pharmaceutical Management Branch, CTEP/DCTD/NCI. Pembrolizumab (MK-3475) injection is a sterile, preservative-free, clear to slightly opalescent, colorless to slightly yellow solution for intravenous use. Each vial contains 100 mg of pembrolizumab (MK-3475) in 4 mL of solution. Each 1 mL of solution contains 25 mg of pembrolizumab (MK-3475) and is formulated in: L-histidine (1.55 mg), polysorbate 80 (0.2 mg), sucrose (70 mg), and Water for Injection, USP.

Preparation: Pembrolizumab (MK-3475) solution for infusion must be diluted prior to administration.. Do not shake the vials. Do not use if opaque or extraneous particulate matter other than translucent to white proteinaceous particles is observed. Do not use if discolored. To prepare the infusion solution add the dose volume of Pembrolizumab (MK-3475) to an infusion bag containing 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP. Gently invert the bag 10-15 times to mix the solution. The final concentration must be between **1 mg/mL to 10 mg/mL**.

Compatible IV bag materials: PVC plasticized with DEHP, non-PVC (polyolefin), EVA, or PE lined polyolefin.

Storage: Store intact vials between 2°C - 8°C (36°F - 46°F). Do not freeze. Protect from light by storing in the original box.

If a storage temperature excursion is identified, promptly return Pembrolizumab (MK-3475) to between 2-8°C and quarantine the supplies. Provide a detailed report of the excursion (including documentation of temperature monitoring and duration of the excursion) to PMBAfterHours@mail.nih.gov for determination of suitability.

Stability: Refer to the package label for expiration.

Administer prepared solutions immediately after preparation. If not administered immediately, prepared solutions may be stored refrigerated for up to 24 hours. Pembrolizumab (MK-3475) solutions may be stored at room temperature for a cumulative time of up to 6 hours. This includes room temperature storage of liquid drug product solution in vials, room temperature storage of infusion solution in the IV bag, and the duration of infusion.

Route of Administration: IV infusion only. Do not administer as an IV push or bolus injection.

Method of Administration: Infuse over approximately 30 minutes (range: 25 - 40 minutes) using an infusion set containing a low-protein binding 0.2 to 5 µm in-line filter made of polyethersulfone or polysulfone. Infusion rate should not exceed 6.7 mL/min. A central line is not required; however, if a subject has a central venous catheter in place, it is recommended that it be used for the infusion. Do not co-administer other drugs through the same infusion line. Following the infusion, flush the IV line with normal saline.

Compatible infusion set materials: PVC plasticized with DEHP or DEHT, PVC and tri-(2-ethylhexyl) trimellitate, polyethylene lined PVC, polyurethane, or polybutadiene

Patient Care Implications: Refer to the protocol for information on evaluation and management of potential immune-related AEs.

Availability

Pembrolizumab (MK-3475) is an investigational agent supplied to investigators by the Division of Cancer Treatment and Diagnosis (DCTD), NCI.

Pembrolizumab (MK-3475) is provided to the NCI under a Collaborative Agreement between the Pharmaceutical Collaborator and the DCTD, NCI (see Section 13.4).

8.1.2 Agent Ordering and Agent Accountability

8.1.2.1 NCI-supplied agents may be requested by eligible participating Investigators (or their authorized designee) at each participating institution. The CTEP-assigned protocol

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

Sites may order initial agent supplies when a subject has been randomized.

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

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

8.1.3 Investigator Brochure Availability

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

8.1.4 Useful Links and Contacts

- CTEP Forms, Templates, Documents: http://ctep.cancer.gov/forms/
- NCI CTEP Investigator Registration: RCRHelpDesk@nih.gov
- PMB policies and guidelines: http://ctep.cancer.gov/branches/pmb/agent_management.htm
- PMB Online Agent Order Processing (OAOP) application: https://ctepcore.nci.nih.gov/OAOP
- CTEP Identity and Access Management (IAM) account: https://ctepcore.nci.nih.gov/iam/
- CTEP IAM account help: ctepreghelp@ctep.nci.nih.gov
- IB Coordinator: IBCoordinator@mail.nih.gov
- PMB email: PMBAfterHours@mail.nih.gov

- PMB phone and hours of service: (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET)

8.2 Commercial Agent(s)

8.2.1 Azacitidine NSC# 102816

Molecular Formula: $C_8H_{12}N_4O_5$

M.W.: 244

Description: White to off-white solid.

How Supplied: The finished product is supplied in a sterile form for reconstitution as a suspension for subcutaneous injection or reconstitution as a solution with further dilution for intravenous infusion. Azacitidine is supplied as a lyophilized powder in 100 mg single-use vials. Store unreconstituted vials at 25° C (77° F); excursions permitted to 15°-30° C (59°-86° F).

Preparation for SubQ: Azacitidine should be reconstituted aseptically with 4 mL sterile water for injection. The diluent should be injected slowly into the vial. Vigorously shake or roll the vial until a uniform suspension is achieved. The suspension will be cloudy. The resulting suspension will contain azacitidine 25 mg/mL. Do not inject the slurry intravenously. Doses greater than 4 mL should be divided equally into 2 syringes, or per institutional standard. The product may be held at room temperature for up to 1 hour, but must be administered within 1 hour after reconstitution.

Storage and Stability for SubQ: The reconstituted product may be kept in the vial or drawn into a syringe. Doses greater than 4 mL should be divided equally into 2 syringes. The product must be refrigerated immediately, and may be held under refrigerated conditions (2°C - 8°C, 36°F - 46°F) for up to 8 hours. After removal from refrigerated conditions, the suspension may be allowed to equilibrate to room temperature for up to 30 minutes prior to administration.

SubQ Administration: To provide a homogeneous suspension, the contents of the dosing syringe must be re-suspended immediately prior to administration. To re-suspend, vigorously roll the syringe between the palms until a uniform, cloudy suspension is achieved. Azacitidine suspension is administered subcutaneously. Doses greater than 4 mL should be divided equally into 2 syringes (or per institutional standard), and injected into 2 separate sites. Rotate sites for each injection (thigh, abdomen, or upper arm). New injections should be given at least one inch from an old site and never into areas where the site is tender, bruised, red, or hard.

Preparation for IV: Reconstitute the appropriate number of vials to achieve the desired dose. Reconstitute each vial with 10 mL sterile water for injection. Vigorously shake or roll the vial until all solids are dissolved. The resulting solution will contain azacitidine 10 mg/mL. Withdraw the required amount of azacitidine to deliver the desired dose and inject into a 50 -100 mL infusion bag of either 0.9% Sodium Chloride Injection or Lactated Ringer's Injection.

Storage and Stability for IV: Azacitidine reconstituted for intravenous administration may be stored at 25°C (77°F), but administration must be completed within 1 hour of reconstitution.

IV Administration: IV infusion over a period of 10-40 minutes.

Agent Ordering: Azacitidine is available from commercial sources.

For more detailed information, please consult the package insert.

8.2.2 Venetoclax NSC#766270

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

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

CAS registry number: 1257044-40-8

Molecular Formula: $C_{45}H_{50}ClN_7O_7S$

M.W.: 868.44 g/mol

Approximate Solubility: very low aqueous solubility

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

Description: Venetoclax tablets for oral administration are supplied as pale yellow or beige tablets that contain 100 mg venetoclax as the active ingredient. Each tablet contains the following inactive ingredient: copovidone, colloidal silicone dioxide, polysorbate 80, sodium stearyl fumarate, and calcium phosphate dibasic. In addition, the 100 mg coated tablets include the following: iron oxide yellow, iron oxide red, iron oxide black, polyvinyl alcohol, talc, polyethylene glycol, and titanium dioxide. Each tablet is embossed with "V" on one side and "100" corresponding to the tablet strength on the other side.

How Supplied: 100 mg tablets for each cycle

Dosing Scheme for Ramp-Up Phase

Day	Venetoclax Daily Dose
1	100 mg
2	200 mg
3	400 mg
4 and beyond	400 mg

Storage: Store at or below 86°F (30°C)
Route(s) **of Administration:** PO

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

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

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

Potential Drug Interactions: Venetoclax is predominantly metabolized by CYP3A4/5. Concomitant use of venetoclax with strong inhibitors of CYP3A at initiation and during ramp-up phase is discouraged. If used during ramp-up, appropriate dose adjustments of venetoclax ramp-up should be followed. For patients who have completed the ramp-up phase and are on a steady daily dose, reduce the dose of venetoclax by at least 75% when used concomitantly with strong CYP3A inhibitors. Resume the venetoclax dose that was used prior to initiating the CYP3A inhibitor or the full venetoclax dose 2 to 3 days after discontinuation of the inhibitor.

Azoles are standard prophylactic and therapeutic anti-fungal agents used in leukemia patients and their use is allowed on trial. When used in patients with AML who are receiving venetoclax, careful monitoring and dose adjustments as per standard clinical care is recommended.

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

When possible., avoid concomitant use with strong CYP3A inducers or moderate CYP3A inducers.

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

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

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

Avoid grapefruit products, Seville oranges, and starfruit during treatment

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

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

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

Nursing women should discontinue breastfeeding during treatment with venetoclax.

Availability

Venetoclax is available from commercial sources.

For more detailed information, please consult the package insert.

9 STATISTICAL CONSIDERATIONS

9.1 Study Design/Endpoints

This is an open label randomized phase 2 study. The primary objective of the phase 2 study is to determine the efficacy of MK3475 (pembrolizumab) in combination with azacitidine and venetoclax in elderly (≥60-year-old) patients with newly diagnosed AML not eligible for intensive chemotherapy. A total of 76 patients will be included (38 patients in the intervention arm and 38 patients in the control arm, see below for sample calculation).

Early Safety assessment: After the first 6 patients have been enrolled on the experimental VAP arm, a safety review committee comprised of the study chair, the PIs of any patient who was enrolled in the experimental arm of the trial, and a representative from CTEP will review the toxicity data to identify any concerning drug-related safety signals before enrolling further patients on the experimental arm. To be considered evaluable for purposes of the early safety rule, the patient must have received one pembrolizumab dose at least. If severe Pembrolizumab -related toxicity was identified in 2 or more of these first 6 patients, the safety review committee will make a recommendation to adjust dosing/schedule or any other changes before enrollment resumes. The evaluability period includes the first 35 days after pembrolizumab first dose, till hematologic recovery, or till starting cycle 2 of therapy, whichever occurs first.

Futility analysis: After 15 patients in each of the two study arms have their primary endpoint results available (per Section 9.5.2), a formal futility analysis will be conducted. Enrollment to study will not be held while the futility analysis is being conducted. If the rate of MRD negative CR (primary efficacy end point) is lower in the experimental arm than the control arm by any amount, the trial would stop for futility.

Toxicity analysis: After 15 patients in each of the two study arms have their primary endpoint results available (per Section 9.5.2), a formal toxicity analysis will be conducted along with futility analysis. If the schedule results in unacceptable toxicity (per Sections 9.4 and 9.5.1), we will discuss alternative approaches with CTEP

For patients who neither relapse nor die, the duration of response (DOR) will be censored on the date of their last evaluable disease assessment. Median relapse-free survival (RFS) and DOR will be estimated with Kaplan-Meier curves with 95% confidence interval calculated based on the Brookmeyer-Crowley method. Long-term patient survival and disease status information will be collected for up to two years from first dose. An analysis of the survival and RFS data will be conducted based on the two-year follow up. Patients alive at the end of the follow-up period or lost to follow-up will be censored at the last date the patient is known to be alive. Continuous variables will be expressed by means, standard deviations and 95% confidence intervals. Frequencies will be computed for discrete data. Bonferroni's correction will be used for multiple comparisons, if any, to protect the overall type I error rate.

9.1.1 Primary endpoint

- Assess the percentage of patients with MRD-CR or MRD-Cri with AZA + VEN with pembrolizumab (MK-3475) during the first 6 cycles of therapy.

9.1.2 MRD assessment by multicolor flow cytometry (MFC) as an integral biomarker

We will use a cutoff of 0.1% as the threshold to distinguish MRD-positive from MRD-"negative" patients as this threshold was found relevant in most published studies to date and was included in the official consensus document on minimal residual disease by the European LeukemiaNet MRD Working Party. However, it should be noted that MRD tests with MRD quantified below <0.1% may still be consistent with residual leukemia, and several studies have shown prognostic significance of MRD levels below 0.1%. Thus, cutoff levels below 0.1% (e.g., <0.01%) may define patients with particularly good outcome.

9.2 Sample Size/Accrual Rate

In a recent study of azacitidine and venetoclax therapy in newly diagnosed elderly AML, a complete response was achieved in 73%, of all patients, of whom 30% attained MRD negativity, thereby yielding approximately 20% MRD- CR rate.(DiNardo et al, 2019). We hypothesize that the addition of pembrolizumab (MK-3475) to AZA+VEN therapy will lead to an improvement of MRD negativity at/after attainment of CR when compared to AZA+VEN therapy alone. Assuming MRD-negative CR/CRi rates of 0.2 and 0.45 for Aza + Ven and Aza + Ven + MK3475 (pembrolizumab), respectively, a total sample of 60 evaluable patients would provide 80% power to detect a between-arm differencing based on a two-sample test of proportions (normal approximation) with one-sided type-I error of 10%. Allowing for up to 20% dropout, the total target accrual would be 76 patients (38 per arm).

PLANNED ENROLLMENT REPORT

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	1	1	1	1	**4**
Asian	1	1	1	1	**4**
Native Hawaiian or Other Pacific Islander	1	1	1	1	**4**
Black or African American	2	3	1	1	**7**
White	20	25	4	4	**53**
More Than One Race	1	1	1	1	**4**
Total	**26**	**32**	**9**	**9**	**76**

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9.3 Stratification Factors

Planned stratification factors include cytogenetics by FISH or karyotype (intermediate/unknown *vs.* adverse karyotype), antecedent hematologic disorder defined as prior MDS, MPN or aplastic anemia (present *vs.* absent), and reason to not receive therapy (ineligibility *vs* refusal). Dose escalation and MTD determination are not parts of this study.

Refer to Section 2.5.1 for further details on assessment of karyotyping.

9.4 Analysis of Secondary Endpoints

Safety Endpoints

Assessment of the proportion of patients who develop severe toxicity, defined as the occurrence of any of the below AE during the first 35 days from start of pembrolizumab (MK-3475) therapy. Severe pembrolizumab-related toxicity is defined as the occurrence of any of the below AEs that are attributed to pembrolizumab (MK-3475). The evaluability period includes the first 35 days after pembrolizumab first dose, till hematologic recovery, or till starting second AZA+Ven cycle therapy, whichever occurs first:

- Any grade 3 or 4 non-hematologic toxicity, that is deemed not related to the underlying disease or chemotherapy, with the following exceptions: a) Transient laboratory abnormalities that can be treated or resolve to grade 2 or less within 7 days, or b) grade 3 expected and known drug-related autoimmune events (such as pneumonitis, enterocolitis, hepatitis, hypophysitis, and skin rash) that resolve within 4 weeks of steroid therapy to grade 1.
- Grade 4 thrombocytopenia or neutropenia associated with aplasia lasting >42 days from the last dose of study drug (pembrolizumab). Given that severe neutropenia and thrombocytopenia are features of AML and commonly encountered in this patient population especially with use of venetoclax, they will not be used to define unacceptable toxicity except if associated with prolonged treatment-associated aplasia AND not associated with active disease that is attributed to pembrolizumab.

- Patients with grade 3 and 4 immune related adverse events (with exception of events that resolve with steroids to grade 1 or less and do not require continued steroid therapy beyond 4 weeks).

9.4.1 Exploratory Endpoints

Descriptive statistics will be provided for each biomarker of interest in overall sample and/or by treatment arms across different time points of the measurements (e.g., baseline, end of induction, post-cycle1, 2, 4, and 6, every 3 month during maintenance, one year, and end of treatment when applicable). Continuous biomarkers (e.g., count of biomarkers from tumor infiltrating lymphocytes, gene expression from RNASeq, Nanostring, and WES, inflammation biomarkers from Olink, DNA methylation, and immune cell expression from CyTOF) will be summarized using mean, standard deviation, median, inter-quartile range, and range. Categorical biomarkers (e. g, mutation, PD-1 status, and tumor mutation burden, microbiome data) will be summarized using frequency counts and percentages. Boxplots, bar plots or variation of boxplots (e.g., swarmplot, violinplot) will be generated for visualization. Distribution of normality of markers will be tested, and transformation of data will be applied if needed. Classification variables may be considered using median, tertile cut-off points, or recursive partitioning methods. A false discovery rate of 5% will be used to account for multiple comparisons and the associated inflation of false detection rates.

Part 1: Assess whether baseline biomarkers or the serial measurements of biomarkers are associated with response outcomes.

A univariate logistic regression will be selected to assess baseline biomarkers associated with response outcomes. The dynamic changes of PD-L1/ PD-1 expressions, concentration of cytokine, and RNA seq/TCRseq, and gut microbiome, etc. will be monitored. The measurements of biomarkers in changes over time from baseline to several time-points will be performed by using generalized linear mixed effects modeling with a Benjamini-Hochberg[1] correction to control for false discovery rates.

Part 2: Assess whether baseline biomarkers or the serial measurements of biomarkers are associated with survival outcomes.
The Kaplan-Meier method and log-rank test will be used to estimate the distribution of survival between/among different marker strata. Univariate or Multivariate cox proportional hazard models will be employed to explore the significance of biomarkers on survival outcomes, while adjusting for the potential prognostic factors. The interaction effects between treatment and biomarkers also will be evaluated. Serial measurements of biomarkers will be estimated at baseline, end of induction, post-cycle1, 2, 4, and 6, every 3 months during maintenance, one year, and end of treatment when applicable. Landmark analysis[1, 2] or joint modeling[3] will be used to assess serial measurements of biomarkers dynamical impacts on survival outcomes, where appropriate.

Part 3: Compare the biomarkers effects between treatment arms, VZA+VEN vs. VAP
The associations between treatment arms and baseline biomarkers will be evaluated using Chi-squared test/ Fisher's exact test, ANOVA and the Mann-Whitney U tests as appropriate. Trajectory trends of the changes in markers' values or status across the measurement time will be explored using generalized linear mixed models. The bar plots and trajectory time plots will visually show the differences over time between treatment arms. The associations between markers and the demographic/prognostic factors will also be assessed using the similar statistical methods.

Part 4: Evaluate the correlations between biomarkers.
The correlations between biomarkers will be evaluated using Pearson/Spearman rank-order correlation coefficients, Chi-squared/Fisher's exact tests, and Wilcoxon rank sun / Kruskal-Wallis tests as appropriate. A scatter plot, boxplot, and mosaic plot will also be generated for visualization. The multiplicity of the endpoints will be adjusted using the correction of Benjamini and Hochberg.

9.4.1.1 MRD assessment by DS as an exploratory biomarker

De-identified DNA samples will be shipped to TwinStrand for DS at an average molecular depth of 20,000x. Samples will initially be investigator-blinded as to any patient information. Library preparation, sequencing, and analysis will be performed with TwinStrand's optimized workflow, and TwinStrand's bioinformatics core will perform all analyses related to assay output.

We anticipate two potential issues using DS:

(1) MRD positive cases without relapse ("false positives"):
It is possible that our assay will not always be able to distinguish true MRD from clonal hematopoiesis of indeterminate potential (CHIP), and misclassification of CHIP as MRD would yield "false positive" results. While some mutations in AML genes (*e.g.*, *KRAS, FLT3*) are pathognomonic for disease, others (*e.g.*, *DNMT3A*) are potentially related to CHIP and can persist in remission without indicating imminent relapse and can even be detected in healthy older individuals. Rather than simply ignoring these mutations, we will use our dataset to see if they can provide prognostic information. If our model does not adequately differentiate between MRD and CHIP based on a high proportion of false positive cases, we will consider alternative

strategies, including having our Fred Hutch collaborators perform flow sorting to isolate lymphoid cells, with the theory that mutations present in both lymphoid and myeloid cells are very likely to be CHIP-related. It should be noted that any sufficiently sensitive MRD assay faces with the same problem of distinguishing biological background from true disease, and this issue is not unique to our novel DS approach. Rather than ignoring the "CHIP problem", however, we will harness the power of a large dataset plus the unprecedented sensitivity of the DS platform to understand how best to analyze all genomic data, CHIP-related and not, to provide prognostic information.

(2) MRD negative cases that relapse ("false negatives"):
One reason for residual disease to be undetectable in bone marrow is hemodilution, or contamination with peripheral blood. We will verify whether hemodilution is present on pathology reports. However, we should also consider the possibility that our assay either does not capture or is insufficiently sensitive for the particular persistent clone(s). Insufficient quantity of DNA is unlikely to lead to false negatives: ~1µg is needed for DS, and we would expect to isolate dozens of micrograms from each sample. In all "false negative" cases, we will perform our DS panel on the patient's marrow sample from diagnosis. For any mutations present at diagnosis, we would plan to repeat sequencing to a greater depth on remission marrows just for this subset. Practically, we would be able to sequence to a depth of 50,000x or 100,000x, which would provide 80% power to detect alleles with frequency of 0.003% (1 in 30,000) or 0.0017% (1 in 60,000) respectively. While we have designed our panel to have one-size-fits-all utility for the vast majority of AML patients, if we find that deeper sequencing reduces false negatives, we could either routinely sequence the entire panel more deeply or, most cost-effectively, simply use only a small subset of the probes to interrogate mutations known to be relevant for a given patient based on diagnosis. It is also possible that rare patients will have mutations that are not picked up in our panel. Our panel, which is relatively small, does not include several larger AML-associated rearrangements. Many commercial products based on RT-PCR are able to detect these rearrangements extremely accurately. For the rare samples where these are the only markers of disease, we could supplement our panel with specific RT-PCR for these fusion products.

9.4.1.1.1 Comparing DS and MFC for MRD detection as an exploratory biomarker

In order to compare both approaches, DS will be performed on these samples at a molecular depth of 20,000x and at the same time the samples will also perform 10-color MFC, although researchers will be blinded to MFC results. The primary analysis will involve comparison in performance of MRD detected by DS *vs*. MFC patients. McNemar's Chi-squared test for paired samples (regular DS *vs*. MFC and ultra-deep DS *vs*. MFC) will be used to compare the MRD measures.

9.4.1.1.2 Analyzing circulating free DNA (cfDNA) as an exploratory biomarker
We will leverage the high sensitivity and accuracy of DS to detect and quantitate low level mutations in circulating free DNA (cfDNA) which will allow for examining the prognostic value of not only circulating tumor DNA (ctDNA) but also specific genes and mutation allele frequencies. Blood samples will be collected at baseline and pre-defined time points after treatment.

The ability to accurately assess ctDNA-based MRD would 1) allow the development and application of DS strategy for ctDNA-based longitudinal MRD monitoring in acute myeloid leukemia. While MRD is known to be a powerful tool to predict relapse, shortcomings in sensitivity and accuracy of conventional MRD techniques such as MFC limits MRD detection and risk assessment. In this regard, the unprecedented sensitivity and accuracy of DS would allow for very low-level detection of MRD and potentially more accurately predict relapse. Second, DS-based ctDNA MRD detection serves as a proof-of-principle study supporting the utility of ctDNA as a clinical biomarker of relapse in hematologic malignancies. ctDNA represents one of the most sensitive and noninvasive biomarkers in solid cancers. However, there far fewer studies exploring its utility in hematologic malignancies. Third, if shown to be an accurate predictor of relapse, ctDNA MRD detection may potentially act as a sensitive and accurate surrogate marker of long-term response in future clinical trials. Finally, ctDNA MRD detection will allow us to monitor disease progression and understand patterns of relapse in the standard and I/O with standard therapy settings, especially addressing if these are different in the setting of I/O directed therapy. Further, ctDNA monitoring can provide insights into clonal tumor hierarchy and capture subclonal dynamics.

The best cell for monitoring MRD is unclear. In chronic myeloid leukemia (CML) studies have shown that peripheral blood monitoring is comparable to bone marrow, and therefore ELN and NCCN guidelines recommend a bone marrow only at diagnosis, with interval monitoring of MRD performed on peripheral blood. Assessment of the bone marrow in the MRD state may be limited by special factors (the biopsy needle misses the residual AML), or dilution (the tube sent for MRD is usually the last one). Thus, peripheral blood may have an advantage of being a collection site for many small clusters of MRD residing in the marrow. AML derived ctDNA may be an advantage over both marrow and peripheral assessment, as it assays discarded DNA from AML cells residing in marrow niches, but not circulating. ctDNA constitutes only a fraction of the total cfDNA pool in the plasma and, therefore, it is very likely that ctDNA occurs at very low allele frequencies. However, ctDNA is an excellent template for sensitive DNA sequencing techniques, as the ctDNA is "pre-sheared" to a uniform fragment size of ~175 bp due to Caspase activity. Not having to ultrasonically fragment the ctDNA, as opposed to normal genomic DNA, improves conversion efficiency several hundred percent. We will leverage the high sensitivity and accuracy of DS to detect and quantitate low level mutations in cfDNA which will allow for examining the prognostic value of not only ctDNA but also specific genes and mutation allele frequencies. Blood samples will be collected at baseline and pre-defined time points after treatment.

To compare cfDNA to both DS-based and MFC serial assessment for relapse prediction, separate multivariate models will be developed for each method. Relapse will be modeled as a time-to-event endpoint and Cox regression will be used to assess the association of ctDNA based MRD with this outcome. In the SWOG study, S0106 for newly diagnosed adult AML, MRD status by MFC was a strong predictor of survival, yet MRD predicted relapse-free survival with a poor c-statistic of 0.58.[1] We hypothesize that DS will lead to an improvement in prediction accuracy of relapse-risk (c-statistic 0.85). Assuming MRD-negativity (among CR/CRi) ratios of 1, 3, 0.3, and 0.45 for intensive chemo, intensive chemo + Pem, Aza + Ven, and Aza + Ven + Pem arms, respectively, and a correlation coefficient (ρ) between MFC and DS assays of 0.75 (based on

MFC and NGS comparison data),[29] a minimum total sample size of 16, 24, 20, and 18 evaluable patients with CR/CRi would provide 80% power to detect a between-arm differencing based on a comparison of two proportional hazards survival curves with one-sided type-I error of 10% without correction for the multiplicity of endpoints: TTR; DFS; and OS.

9.4.1.2 Assessment of cytokine panel

For data analysis, IFN-γ^+/CD3$^+$/ CD4$^+$ or IFN-γ^+/CD3$^+$/CD8$^+$ events will be gated and percentages of the total CD4$^+$ and CD8$^+$ T cells will be determined, although these may change as the technology evolves and develops. Percentages of IFN-γ^+ events in corresponding gates from un-stimulated control samples will be subtracted. This analysis will be done both at day 30 (after administration of pembrolizumab and count recovery) and after cycles 2,4, and 6 of therapy to compare levels of leukemia specific T-cell prior to and after anti-PD-L1 therapy.

9.4.1.3 Evaluate whether the expression of PD-1, PD-L1 in AML BM correlates with the response to anti-PD-1 therapy and characterize dynamic change of immune cell subsets in AML patients pre- and post- anti-PD-1 therapy:

Multiplex immunofluorescent assay (IF) will be performed in samples collected at the beginning of trial, post cycle 4, post cycle 6, after 1 year, at relapse, and at end of treatment/progression using PD-L1 (clone: E1L3N, Cell Signaling Technology) antibody and PD-1 (clone: EH33, Cell Signaling Technology) antibody. We will assess PD-L1 expression and PD-1 expression in CD34$^+$ or CD33$^+$ population (depending on initial phenotype of AML blasts).

An association of clinical response with the expression of PD-L1 AML BM cells will be assessed by a Pearson chi-square test on a 2x2 table of frequencies. The dependent variable will be defined as response (yes versus no), and QIF categories (negative *vs.* positive) will be the independent variables. We will also monitor the dynamic change of PD-L1 expression over the course of treatment and its correlation with clinical response. Longitudinal measurements of PD-L1 will be examined using mixed-effects modeling.

9.4.1.4 Dynamic change of immune subsets

We will assess these immune cell markers at different time points of therapy: at baseline (prior to application of AZA+VEN therapy), at Day 30 (at time of count recovery), as well as after cycles 2, 4, and 6 of therapy. We also plan to measure CD47 levels on leukemic blasts prior to and after AZA+VEN therapy and pembrolizumab (MK-3475) application to see whether CD47 expression levels correlate with responders *vs*. non-responders and whether expression levels change at different times of therapy. Statistical analyses of the frequency of CD8$^+$, CD4$^+$, Foxp3 T_{regs}, CD8$^+$/Foxp3$^+$ T_{regs}, $T_{CM}/T_{EMRA,}$ $T_{EM}/T_{EMRA,}$ the percentage of Ki67 and GzmB in PD-1+, Eomes$^+$ CD8 T cells to compare changes over time from baseline to several time-points will be performed by using mixed effects modeling with a Benjamini-Hochberg correction to control for false discovery rates

9.4.1.5 .. Correlation between microbial changes with immune-checkpoint expression and kinetics of immune cell subset recovery and programming

Analysis of microbiome communities will be performed in R (http://www.R-project.org), using phyloseq27 to calculate α- and β- diversity metrics. The Shannon Diversity Index (SDI) will be used for α-diversity (variance within a particular sample; a measure of bacterial diversity) metric calculations, and weighted and unweighted UniFrac for β-diversity distances (characterization of differences in microbial community composition; a measure of temporal stability of the microbial community structure).28 Temporal variability will be determined using coefficients of variation (CV) [a measure of stability of species diversity over time] of the SDI and unweighted and weighted UniFrac distances per patient, with higher values indicative of more variable community composition. Pairwise differences in temporal variability across body sites will be made using Mann–Whitney U test, whereas pairwise differences among response groups performed using Student's t-test. Linear correlations between CVs will be determined using Pearson's r correlation. The population cohort will be divided into quartiles based on CV of the weighted UniFrac distance values or SDI where the first quartile is defined as stable, second and third as average, and fourth as variable. Measurements of intra-patient temporal variability and patient stability categories will be analyzed for their correlations with genera abundances. Groups of patients will be analyzed to determine if patients with subsequent clinical outcomes had significantly different levels of microbiome temporal variability. Potential clinical drivers of microbiome temporal instability will be determined using multivariable regression analyses. Untargeted metabolite analyses from stool samples with mass spectroscopy will be performed. The primary outcome of interest is to longitudinally characterize microbial shifts in patients with AML receiving PD-1 inhibitors. For this analysis standard of care will be compared with the experimental arm which includes chemotherapy + pembrolizumab (MK-3475) combination. Secondary outcomes of interest will be a measure of both efficacy and toxicity which will include variables like rates of CR/CRi, progression free survival (PFS), overall survival (OS), cumulative incidence of graft versus host disease (GVHD)/non-relapse mortality (NRM). Association between microbial diversity on the percentage of patients with minimal residual disease (MRD) negative complete remission (CR) (MRD-CR) as measured by flow cytometry at the end of six cycles will also be explored

9.5 For phase 2 protocols only: Reporting and Exclusions

9.5.1 Evaluation of Toxicity

All patients will be evaluable for toxicity from the time of their first treatment with pembrolizumab (MK-3475).

Severe pembrolizumab-related toxicity is defined as the occurrence of any of the below AEs that are attributed to pembrolizumab (MK-3475). The evaluability period includes the first 35 days after pembrolizumab first dose, till hematologic recovery, or till starting second AZA+Ven cycle therapy, whichever occurs first:

- Any grade 3 or 4 non-hematologic toxicity, that is deemed not related to the underlying disease or chemotherapy with the following exceptions: a) transient laboratory

abnormalities that can be treated or resolve to grade 2 or less within 7 days; or b) grade 3 expected and known drug-related autoimmune events (such as pneumonitis, enterocolitis, hepatitis, hypophysitis, and skin rash) that resolve within 4 weeks of steroid therapy to grade 1.

- Grade 4 thrombocytopenia or neutropenia, associated with aplasia lasting >42 days from the last dose of study drug (pembrolizumab). Given that severe neutropenia and thrombocytopenia are features of AML and commonly encountered in this patient population especially with use of venetoclax, they will not be used to define unacceptable toxicity except if associated with prolonged treatment-associated aplasia AND not associated with active disease that is attributed to pembrolizumab.

- Patients with grade 3 and 4 irAEs (with exception of events that resolve with steroids to grade 1 or less and do not require continued steroid therapy beyond 4 weeks).

9.5.2 Evaluation of Response

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

All of the patients who met the eligibility criteria (with the possible exception of those who received no study medication) should be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific.

All conclusions should be based on all eligible patients. Sub-analyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (*e.g.*, early death due to other reasons, early discontinuation of treatment, major protocol violations, *etc.*). However, these subanalyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported. The 95% confidence intervals should also be provided.

9.6 Data Safety Monitoring Board

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

10 ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

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

10.1 Comprehensive Adverse Events and Potential Risks List(s) (CAEPRs)

The Comprehensive Adverse Events and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification.
NOTE: Report AEs on the SPEER ONLY IF they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

10.1.1 CAEPRs for CTEP IND Agent

10.1.1.1 CAEPR for pembrolizumab (MK-3475)

Comprehensive Adverse Events and Potential Risks list (CAEPR)
for
Pembrolizumab (MK-3475, NSC 776864)

The Comprehensive Adverse Events and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 3793 patients.* Below is the CAEPR for Pembrolizumab (MK-3475).

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

Version 2.8, August 14, 2024[1]

Adverse Events with Possible Relationship to Pembrolizumab (MK-3475) (CTCAE 5.0 Term) [n= 3793]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia[2]		
		Blood and lymphatic system disorders - Other (immune thrombocytopenic purpura)[2]	
		Blood and lymphatic system disorders - Other (autoimmune hemolytic anemia)[2]	
	Lymph node pain[2]		
CARDIAC DISORDERS			
		Myocarditis[2]	
		Pericarditis[2]	
ENDOCRINE DISORDERS			
	Adrenal insufficiency[2]		
		Endocrine disorders - Other (hypoparathyroidism)[2]	
	Endocrine disorders - Other (thyroiditis)[2]		
	Hyperthyroidism[2]		
	Hypophysitis[2]		
	Hypopituitarism[2]		
	Hypothyroidism[2]		
EYE DISORDERS			
		Eye disorders - Other (Vogt-Koyanagi-Harada syndrome)	
		Uveitis[2]	
GASTROINTESTINAL DISORDERS			
	Abdominal pain		
	Colitis[2]		
	Diarrhea[2]		***Diarrhea[2] (Gr 2)***
		Enterocolitis[2]	
		Gastritis[2]	
		Gastrointestinal disorders - Other (exocrine pancreatic insufficiency)	
	Mucositis oral[2]		
	Nausea		***Nausea (Gr 2)***
	Pancreatitis[2]		
	Small intestinal mucositis[2]		
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Chills		
Fatigue			***Fatigue (Gr 2)***
	Fever[2]		

Adverse Events with Possible Relationship to Pembrolizumab (MK-3475) (CTCAE 5.0 Term) [n= 3793]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
HEPATOBILIARY DISORDERS			
	Hepatobiliary disorders - Other (autoimmune hepatitis)[2]		
		Hepatobiliary disorders - Other (sclerosing cholangitis)	
IMMUNE SYSTEM DISORDERS			
		Anaphylaxis[2]	
		Cytokine release syndrome[2]	
		Immune system disorders - Other (acute graft-versus-host-disease)[2,3]	
		Immune system disorders - Other (hemophagocytic lymphohistiocytosis)[2]	
	Immune system disorders - Other (sarcoidosis)[2]		
		Serum sickness[2]	
INFECTIONS AND INFESTATIONS			
		Myelitis[2]	
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			
	Infusion related reaction		
INVESTIGATIONS			
	Alanine aminotransferase increased[2]		
	Alkaline phosphatase increased		
	Aspartate aminotransferase increased[2]		
	Blood bilirubin increased		
		GGT increased	
		Lipase increased	
		Serum amylase increased	
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		
	Hyponatremia		
		Metabolism and nutrition disorders - Other (diabetic ketoacidosis)[2]	
		Metabolism and nutrition disorders - Other (type 1 diabetes mellitus)[2]	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia[2]		***Arthralgia [2] (Gr 2)***
	Arthritis[2]		
	Back pain		
	Joint range of motion decreased		
	Myalgia[2]		
	Myositis[2]		
NERVOUS SYSTEM DISORDERS			

Adverse Events with Possible Relationship to Pembrolizumab (MK-3475) (CTCAE 5.0 Term) [n= 3793]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
		Guillain-Barre syndrome[2]	
		Myasthenia gravis	
		Nervous system disorders - Other (autoimmune neuropathy)[2]	
		Nervous system disorders - Other (demyelination)[2]	
		Nervous system disorders - Other (myasthenic syndrome)[2]	
		Nervous system disorders - Other (nerve paresis)[2]	
		Nervous system disorders - Other (neuromyopathy)[2]	
		Nervous system disorders - Other (non-infectious encephalitis)[2]	
		Nervous system disorders - Other (non-infectious meningitis)[2]	
		Nervous system disorders - Other (non-infectious myelitis)	
		Nervous system disorders - Other (optic neuritis)	
		Nervous system disorders - Other (polyneuropathy)[2]	
		Paresthesia	
		Peripheral motor neuropathy[2]	
RENAL AND URINARY DISORDERS			
		Acute kidney injury	
		Renal and urinary disorders - Other (autoimmune nephritis)[2]	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
		Pneumonitis[2]	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Bullous dermatitis[2]		
		Erythema multiforme[2]	
	Erythroderma		
		Palmar-plantar erythrodysesthesia syndrome	
	Pruritus[2]		***Pruritus[2] (Gr 2)***
	Rash acneiform[2]		
	Rash maculo-papular[2]		***Rash maculo-papular[2] (Gr 2)***
		Skin and subcutaneous tissue disorders - Other (Drug reaction with eosinophilia with systemic symptoms [DRESS])[2]	
	Skin and subcutaneous tissue disorders - Other (dermatitis)[2]		
	Skin hypopigmentation[2]		
		Stevens-Johnson syndrome[2]	

Adverse Events with Possible Relationship to Pembrolizumab (MK-3475) (CTCAE 5.0 Term) [n= 3793]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
		Toxic epidermal necrolysis[2]	
	Urticaria[2]		
VASCULAR DISORDERS			
		Vasculitis[2]	

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

[2]Immune-mediated adverse reactions have been reported in patients receiving Pembrolizumab (MK-3475). Adverse events potentially related to Pembrolizumab (MK-3475) may be manifestations of immune-mediated adverse events. In clinical trials, most immune-mediated adverse reactions were reversible and managed with interruptions of Pembrolizumab (MK-3475), administration of corticosteroids and supportive care.

[3]Acute graft-versus-host disease has been observed in patients treated with Pembrolizumab (MK-3475) who received hematopoeitic stem cell transplants.

Adverse events reported on Pembrolizumab (MK-3475) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that Pembrolizumab (MK-3475) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (pancytopenia); Disseminated intravascular coagulation
CARDIAC DISORDERS - Atrial fibrillation; Cardiac arrest; Chest pain - cardiac; Heart failure; Myocardial infarction; Pericardial effusion; Pericardial tamponade; Ventricular arrhythmia
EYE DISORDERS - Eye pain
GASTROINTESTINAL DISORDERS - Abdominal distension; Ascites; Constipation; Duodenal hemorrhage; Dysphagia; Gastrointestinal disorders - Other (intussusception); Gastrointestinal disorders - Other (diverticulitis); Gastrointestinal disorders - Other (intestinal obstruction); Oral pain; Rectal hemorrhage; Small intestinal perforation; Upper gastrointestinal hemorrhage; Vomiting
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Edema face; Edema limbs; Facial pain; Gait disturbance; General disorders and administration site conditions - Other (general physical health deterioration); Generalized edema; Malaise; Non-cardiac chest pain; Pain
INVESTIGATIONS - CPK increased; Cholesterol high; Creatinine increased; Fibrinogen decreased; Lymphocyte count decreased; Neutrophil count decreased; Platelet count decreased; Weight loss; White blood cell decreased
METABOLISM AND NUTRITION DISORDERS - Dehydration; Hypercalcemia; Hyperglycemia; Hyperkalemia; Hypertriglyceridemia; Hyperuricemia; Hypoalbuminemia; Hypokalemia; Hypophosphatemia; Metabolism and nutrition disorders - Other (failure to thrive); Tumor lysis syndrome
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Bone pain; Generalized muscle weakness; Joint effusion[2]; Musculoskeletal and connective tissue disorder - Other (groin pain); Pain in extremity
NERVOUS SYSTEM DISORDERS - Aphonia; Depressed level of consciousness; Dysarthria; Edema cerebral; Encephalopathy; Headache; Hydrocephalus; Lethargy; Meningismus; Nervous system disorders - Other (brainstem herniation); Seizure; Syncope; Tremor
PSYCHIATRIC DISORDERS - Agitation; Confusion
RENAL AND URINARY DISORDERS - Nephrotic syndrome; Proteinuria; Renal and urinary disorders - Other (hydronephrosis); Urinary incontinence; Urinary tract pain
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Pelvic pain
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Cough; Dyspnea; Hypoxia; Laryngeal inflammation; Pleural effusion; Pleuritic pain[2]; Pneumothorax; Respiratory failure
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Dry skin; Skin and subcutaneous tissue disorders - Other (drug eruption)
VASCULAR DISORDERS - Hypertension; Peripheral ischemia; Thromboembolic event

Note: Pembrolizumab (MK-3475) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

10.1.1.2 CAEPR for azacitidine

Comprehensive Adverse Events and Potential Risks list (CAEPR) for Azacitidine (NSC 102816)

The Comprehensive Adverse Events and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 1800 patients.* Below is the CAEPR for Azacitidine.

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

Version 2.7, July 30, 2019[1]

Adverse Events with Possible Relationship to Azacitidine (CTCAE 5.0 Term) [n= 1800]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Anemia			***Anemia (Gr 3)***
	Febrile neutropenia		***Febrile neutropenia (Gr 3)***
CARDIAC DISORDERS			
	Heart failure		***Heart failure (Gr 2)***
	Pericardial effusion		***Pericardial effusion (Gr 2)***
	Sinus tachycardia		***Sinus tachycardia (Gr 2)***
	Supraventricular tachycardia		***Supraventricular tachycardia (Gr 2)***
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr 3)***
	Colitis		***Colitis (Gr 2)***
Constipation			***Constipation (Gr 2)***
Diarrhea			***Diarrhea (Gr 3)***
	Esophagitis		***Esophagitis (Gr 2)***
	Gastrointestinal hemorrhage[2]		
	Mucositis oral		***Mucositis oral (Gr 2)***
Nausea			***Nausea (Gr 3)***
Vomiting			***Vomiting (Gr 3)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Chills		***Chills (Gr 2)***
	Edema limbs		***Edema limbs (Gr 2)***
Fatigue			***Fatigue (Gr 3)***

Fever			***Fever (Gr 3)***
Injection site reaction			***Injection site reaction (Gr 2)***

Adverse Events with Possible Relationship to Azacitidine (CTCAE 5.0 Term) [n= 1800]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
IMMUNE SYSTEM DISORDERS			
		Allergic reaction	***Allergic reaction (Gr 2)***
		Anaphylaxis	
INFECTIONS AND INFESTATIONS			
Infection[3]			***Infection[3] (Gr 4)***
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			
	Bruising		***Bruising (Gr 2)***
INVESTIGATIONS			
	Alanine aminotransferase increased		***Alanine aminotransferase increased (Gr 4)***
	Alkaline phosphatase increased		***Alkaline phosphatase increased (Gr 2)***
	Aspartate aminotransferase increased		***Aspartate aminotransferase increased (Gr 4)***
	Blood bilirubin increased		***Blood bilirubin increased (Gr 2)***
	GGT increased		***GGT increased (Gr 2)***
	Lymphocyte count decreased		***Lymphocyte count decreased (Gr 4)***
Neutrophil count decreased			***Neutrophil count decreased (Gr 4)***
Platelet count decreased			***Platelet count decreased (Gr 4)***
	Weight loss		***Weight loss (Gr 2)***
	White blood cell decreased		***White blood cell decreased (Gr 4)***
METABOLISM AND NUTRITION DISORDERS			
	Acidosis		***Acidosis (Gr 2)***
	Anorexia		***Anorexia (Gr 3)***
	Hypokalemia		
		Tumor lysis syndrome	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		***Arthralgia (Gr 2)***
	Back pain		***Back pain (Gr 2)***
	Generalized muscle weakness		***Generalized muscle weakness (Gr 2)***
	Myalgia		***Myalgia (Gr 2)***
	Pain in extremity		***Pain in extremity (Gr 2)***
NERVOUS SYSTEM DISORDERS			
	Dizziness		***Dizziness (Gr 2)***
	Headache		***Headache (Gr 2)***
	Peripheral motor neuropathy		***Peripheral motor neuropathy (Gr 2)***
	Somnolence		***Somnolence (Gr 2)***
PSYCHIATRIC DISORDERS			
	Anxiety		
	Confusion		***Confusion (Gr 2)***
	Insomnia		
RENAL AND URINARY DISORDERS			
		Acute kidney injury	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			

Adverse Events with Possible Relationship to Azacitidine (CTCAE 5.0 Term) [n= 1800]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
		Bronchopulmonary hemorrhage	
	Cough		***Cough (Gr 2)***
	Dyspnea		***Dyspnea (Gr 4)***
	Epistaxis		***Epistaxis (Gr 2)***
	Pharyngolaryngeal pain		
	Postnasal drip		***Postnasal drip (Gr 2)***
	Respiratory, thoracic and mediastinal disorders - Other (abnormal breath sound)[4]		***Respiratory, thoracic and mediastinal disorders - Other (abnormal breath sound)[4] (Gr 2)***
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Alopecia		***Alopecia (Gr 2)***
	Pruritus		***Pruritus (Gr 2)***
	Purpura		***Purpura (Gr 2)***
	Rash maculo-papular		***Rash maculo-papular (Gr 3)***
VASCULAR DISORDERS			
	Hematoma		***Hematoma (Gr 2)***
	Hypotension		***Hypotension (Gr 3)***

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

[2]Gastrointestinal hemorrhage includes Anal hemorrhage, Cecal hemorrhage, Colonic hemorrhage, Duodenal hemorrhage, Esophageal hemorrhage, Esophageal varices hemorrhage, Gastric hemorrhage, Hemorrhoidal hemorrhage, Ileal hemorrhage, Intra-abdominal hemorrhage, Jejunal hemorrhage, Lower gastrointestinal hemorrhage, Oral hemorrhage, Pancreatic hemorrhage, Rectal hemorrhage, Retroperitoneal hemorrhage, and Upper gastrointestinal hemorrhage under the GASTROINTESTINAL DISORDERS SOC.

[3]Infection may include any of the 75 infection sites under the INFECTIONS AND INFESTATIONS SOC.

[4]Abnormal breath sounds include rales and rhonchi.

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

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (agranulocytosis); Blood and lymphatic system disorders - Other (lymphadenopathy); Blood and lymphatic system disorders - Other (pancytopenia); Blood and lymphatic system disorders - Other (splenomegaly); Blood and lymphatic system disorders - Other (transfusion: platelets); Bone marrow hypocellular; Hemolysis; Leukocytosis
CARDIAC DISORDERS - Atrial fibrillation; Atrial flutter; Atrioventricular block complete; Cardiac arrest; Cardiac disorders - Other (cardiac valve vegetation); Cardiac disorders - Other (Wolff-Parkinson-White syndrome); Chest pain - cardiac; Myocardial infarction; Palpitations; Pericarditis; Restrictive cardiomyopathy; Sinus bradycardia; Ventricular fibrillation
EAR AND LABYRINTH DISORDERS - Hearing impaired; Tinnitus

EYE DISORDERS - Eye disorders - Other (eye/conjunctival hemorrhage); Eye disorders - Other (retina hemorrhage); Papilledema; Uveitis
GASTROINTESTINAL DISORDERS - Abdominal distension; Ascites; Duodenal ulcer; Dyspepsia; Dysphagia; Enterocolitis; Esophageal pain; Esophageal ulcer; Flatulence; Gastritis; Gastrointestinal disorders - Other (enteritis); Gastrointestinal disorders - Other (inguinal hernia, obstructive); Gastrointestinal disorders - Other (intestinal ischemia); Gastrointestinal disorders - Other (intussusception); Gastrointestinal pain; Hemorrhoids; Pancreatitis; Periodontal disease; Small intestinal obstruction; Visceral arterial ischemia
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Death NOS; Edema face; Flu like symptoms; Gait disturbance; General disorders and administration site conditions - Other (systemic inflammatory response syndrome); Generalized edema; Malaise; Multi-organ failure; Non-cardiac chest pain; Pain; Sudden death NOS
HEPATOBILIARY DISORDERS - Cholecystitis; Hepatic failure; Hepatobiliary disorders - Other (bile duct stone); Hepatobiliary disorders - Other (hepatic cirrhosis)
IMMUNE SYSTEM DISORDERS - Autoimmune disorder; Immune system disorders - Other (GVHD)
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Burn; Fall; Fracture; Hip fracture; Injury, poisoning and procedural complications - Other (excoriation); Injury, poisoning and procedural complications - Other (transfusion reaction); Postoperative hemorrhage; Wound dehiscence
INVESTIGATIONS - Activated partial thromboplastin time prolonged; Blood lactate dehydrogenase increased; Creatinine increased; Electrocardiogram QT corrected interval prolonged; INR increased; Investigations - Other (blood urea increased); Investigations - Other (cardiac murmur); Investigations - Other (coagulopathy); Investigations - Other (protein total decreased); Investigations - Other (thrombocytosis); Lipase increased; Lymphocyte count increased; Serum amylase increased
METABOLISM AND NUTRITION DISORDERS - Dehydration; Hyperglycemia; Hyperkalemia; Hyperphosphatemia; Hyperuricemia; Hypoalbuminemia; Hypocalcemia; Hypomagnesemia; Hyponatremia; Hypophosphatemia; Metabolism and nutrition disorders - Other (gout exacerbation); Metabolism and nutrition disorders - Other (hypovolemia); Metabolism and nutrition disorders - Other (low carbon dioxide)
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthritis; Bone pain; Chest wall pain; Flank pain; Muscle cramp; Muscle weakness lower limb; Musculoskeletal and connective tissue disorder - Other (chondritis); Musculoskeletal and connective tissue disorder - Other (intervertebral disc protrusion); Musculoskeletal and connective tissue disorder - Other (musculoskeletal stiffness); Neck pain
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Myelodysplastic syndrome; Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (colonic polyp, vaginal polyp); Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (metastases to central nervous system); Treatment related secondary malignancy
NERVOUS SYSTEM DISORDERS - Dysesthesia; Dysgeusia; Hydrocephalus; Intracranial hemorrhage; Lethargy; Memory impairment; Nervous system disorders - Other (head injury); Paresthesia; Peripheral sensory neuropathy; Seizure; Stroke; Syncope
PSYCHIATRIC DISORDERS - Delirium; Depression; Hallucinations; Psychiatric disorders - Other (mental status changes)
RENAL AND URINARY DISORDERS - Chronic kidney disease; Dysuria; Hematuria; Proteinuria; Renal and urinary disorders - Other (bladder distention); Renal and urinary disorders - Other (calculus urinary); Renal calculi; Urinary frequency; Urinary retention
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Erectile dysfunction; Reproductive system and breast disorders - Other (benign prostatic hyperplasia); Uterine hemorrhage; Vaginal hemorrhage
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Atelectasis; Hypoxia; Laryngeal hemorrhage; Nasal congestion; Oropharyngeal pain; Pleural effusion; Pleuritic pain; Pneumonitis; Pneumothorax; Productive cough; Pulmonary edema; Respiratory failure; Respiratory, thoracic and mediastinal disorders - Other (chronic obstructive pulmonary disease); Respiratory, thoracic and mediastinal disorders - Other (pharyngeal erythema); Rhinorrhea; Sinus pain; Wheezing
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Dry skin; Hyperhidrosis; Palmar-plantar erythrodysesthesia syndrome; Skin and subcutaneous tissue disorders - Other (skin laceration); Skin and subcutaneous tissue disorders - Other (skin lesion); Skin and subcutaneous tissue disorders - Other (skin nodule); Skin and subcutaneous tissue disorders - Other (Sweet's Syndrome); Skin induration; Urticaria

VASCULAR DISORDERS - Flushing; Hypertension; Thromboembolic event; Vascular disorders - Other (pallor); Vascular disorders - Other (poor venous access); Vasculitis

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

10.1.1.3 CAEPR for venetoclax

. *Frequency is provided based on 1298 patients.* Below is the CAEPR for Venetoclax (ABT-199).

Version 2.1, May 8, 2019

Adverse Events with Possible Relationship to Venetoclax (ABT-199) (CTCAE 5.0 Term) [n= 1298]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Anemia			***Anemia (Gr 2)***
	Febrile neutropenia		
GASTROINTESTINAL DISORDERS			
	Constipation		***Constipation (Gr 2)***
Diarrhea			***Diarrhea (Gr 2)***
Nausea			***Nausea (Gr 2)***
	Vomiting		***Vomiting (Gr 2)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
Fatigue			***Fatigue (Gr 2)***
	Fever		***Fever (Gr 2)***
NFECTIONS AND INFESTATIONS			
Infection[2]			***Infection[2] (Gr 2)***
NVESTIGATIONS			
	Lymphocyte count decreased		
Neutrophil count decreased			***Neutrophil count decreased (Gr 2)***
	Platelet count decreased		***Platelet count decreased (Gr 2)***
	White blood cell decreased		
METABOLISM AND NUTRITION DISORDERS			
	Hypocalcemia		
	Hypokalemia		***Hypokalemia (Gr 2)***

Adverse Events with Possible Relationship to Venetoclax (ABT-199) (CTCAE 5.0 Term) [n= 1298]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Hypophosphatemia		
		Tumor lysis syndrome	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		
NERVOUS SYSTEM DISORDERS			
	Headache		***Headache (Gr 2)***
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Cough		***Cough (Gr 2)***
VASCULAR DISORDERS			
	Hypertension		

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

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

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

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Hemolysis; Thrombotic thrombocytopenic purpura
CARDIAC DISORDERS - Atrial fibrillation; Cardiac disorders - Other (coronary artery disease); Heart failure; Myocardial infarction; Sinus tachycardia; Ventricular arrhythmia
EAR AND LABYRINTH DISORDERS - Tinnitus; Vertigo
GASTROINTESTINAL DISORDERS - Abdominal pain; Belching; Dry mouth; Dyspepsia; Dysphagia; Gastrointestinal disorders - Other (Crohn's disease); Small intestinal obstruction
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Death NOS; Edema limbs; Flu like symptoms; General disorders and administration site conditions - Other (general physical heath deterioration); General disorders and administration site conditions - Other (multiple organ dysfunction syndrome); Injection site reaction; Non-cardiac chest pain; Pain; Sudden death NOS
HEPATOBILIARY DISORDERS - Hepatobiliary disorders - Other (hepatic function abnormal)
IMMUNE SYSTEM DISORDERS - Cytokine release syndrome
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Infusion related reaction; Injury, poisoning and procedural complications - Other (laceration)
INVESTIGATIONS - Aspartate aminotransferase increased; Blood bilirubin increased; Weight loss
METABOLISM AND NUTRITION DISORDERS - Anorexia; Dehydration; Hyperglycemia; Hyperkalemia; Hyperphosphatemia; Hyperuricemia; Hypoalbuminemia; Hypomagnesemia; Hyponatremia; Metabolism and nutrition disorders - Other (failure to thrive)
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Back pain; Bone pain
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Myelodysplastic syndrome; Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (acute myeloid leukemia); Treatment related secondary malignancy

NERVOUS SYSTEM DISORDERS - Dizziness; Dysesthesia; Intracranial hemorrhage; Ischemia cerebrovascular; Nervous system disorders - Other (neuropathy peripheral); Peripheral sensory neuropathy; Syncope
PSYCHIATRIC DISORDERS - Confusion; Insomnia
RENAL AND URINARY DISORDERS - Acute kidney injury; Cystitis noninfective; Dysuria
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Ovarian rupture
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Allergic rhinitis; Aspiration; Dyspnea; Epistaxis; Hypoxia; Nasal congestion; Oropharyngeal pain; Pleural effusion; Respiratory failure; Respiratory, thoracic and mediastinal disorders - Other (asphyxia)
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Eczema; Pruritus; Rash acneiform; Rash maculo-papular
VASCULAR DISORDERS - Hypotension; Thromboembolic event

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

10.1.2 Adverse Effects of Dexamethasone

Routes: Intraocular; Ophthalmic; Oral; Topical

Common Adverse Effects

- **Cardiovascular:** Hypertension (Diabetic macular edema, 13%)
- **Dermatologic:** Atrophic condition of skin, Finding of skin healing, Impaired
- **Endocrine metabolic:** Cushing's syndrome, Decreased body growth
- **Immunologic:** At risk for infection
- **Ophthalmic:** Abnormal vision (9%), Cataract (Diabetic macular edema, 68%; retinal vein occlusion and uveitis, 5%), Conjunctival edema (5%), Conjunctivitis (6%), Discomfort, Eye (10%), Dry eye syndrome (5%), Raised intraocular pressure (Diabetic macular edema, 35%; retinal vein occlusion and uveitis, 25%), Vitreous floaters (5%)
- **Psychiatric:** Depression, Euphoria
- **Respiratory:** Pulmonary tuberculosis

For complete information on potential AEs for dexamethasone, please refer to Dexamethasone Prescribing Information (Roxane Laboratories, Inc., 2015).

10.1.3 Unknown or Potential Risks Associated with the Use of Pembrolizumab (MK-3475) in AML Therapy

Immune check point blockade studies have demonstrated the feasibility and safety of combining immunotherapy with intensive chemoinduction (Ravandi *et al.,* 2019) and azacitidine (Daver *et al.,* 2018) in patients with AML. In a single-arm phase II part of a phase I/II study, 44 patients aged 18–60 years (>60 years if eligible for intensive chemotherapy) with newly diagnosed AML (n=42) or high-risk myelodysplastic syndrome (n=2) treatment included a 1.5g/m^2, 24-hour infusion of cytarabine daily on Days 1–4 (three days only for patients >60 years), alongside

12mg/m^2 daily on days 1–3 of idarubicin. Nivolumab was given on Day 24 at a dose of 3mg/kg which was repeated every two weeks for a year in responders. Adverse events are detailed in the table below.

Adverse events regardless of causality

	Grade 1-2, n (%)	Grade 3, n (%)	Grade 4, n (%)
Nausea	1 (2)	1 (2)	0
Diarrhea	3 (7)	7 (16)	0
Mucositis or stomatitis	1 (2)	0	0
Muscle weakness	0	1 (2)	0
Syncope	0	1 (2)	0
Elevated transaminases	3 (5)	1 (2)	0
Elevated bilirubin	0	1 (2)	0
Febrile Neutropenia	1 (2)	13 (30)	1 (2)
Rash	1 (2)	2 (5)	0
Pneumonitis	1 (2)	0	0
Colitis	1 (2)	1 (2)	1 (2)
Pancreatitis	1 (2)	1 (2)	0
Cholecystitis	0	1 (2)	0
Thrombosis or embolism	1 (2)	0	0

There were no nivolumab treatment-associated deaths and post-transplant severe GVHD was not significantly increased and manageable.

Similarly, Daver et al reported on the safety outcomes of combining nivolumab with azacitidine in patients with R/R AML. In the phase 2 study enrolling 70 patients, grade 3-4 and grade 2 immune related adverse events (irAEs) were observed in 8 (11%) and 8 (11%) patients, respectively. Of the 16 (23%) patients with grade 2-4 immune toxicities, 9 episodes were pneumonitis, 6 were nephritis, 3 were immune related skin rash, and 2 were transaminitis (some patients had more than 1 irAE). Fourteen of the 16 (88%) toxicities responded to steroids, and these 14 patients were safely rechallenged with nivolumab. The non-immune toxicities with this combination were similar to other HMA-based salvage therapies.

10.2 Adverse Event Characteristics

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

- **For expedited reporting purposes only:**

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

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

10.3 Expedited Adverse Event Reporting

10.3.1 RAVE CTEP-AERS Integration

The Cancer Therapy Evaluation Program Adverse Event Reporting System (CTEP-AERS) integration enables evaluation of post-baseline AEs entered in Rave to determine whether they require expedited reporting, and facilitates entry in CTEP-AERS for those AEs requiring expedited reporting.

All AEs that occur after baseline are collected in Medidata Rave using the Adverse Event form, which is available for entry at each treatment or reporting period, and used to collect AEs that start during the period or persist from the previous reporting period. The Clinical Research Associate (CRA) will enter AEs that occur prior to the start of treatment on a baseline form that is not included in the Rave-CTEP-AERS integration. AEs that occur prior to enrollment must begin and end on the baseline Adverse Event form and should not be included on the standard Adverse Events form that is available at treatment unless there has been an increase in grade.

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

- The reporting period (course/cycle) is correct, and
- AEs are recorded and complete (no missing fields) and the form is query-free (fields added to the form during study build do not need to be query-free for the integration call with CTEP-AERS to be a success).

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

Upon completion of AE entry in Medidata Rave, the CRA submits the AE for rules evaluation by completing the Expedited Reporting Evaluation form. Both NCI and protocol-specific reporting rules evaluate the AEs submitted for expedited reporting. A report is initiated in CTEP-AERS using information entered in Medidata Rave for AEs

that meet reporting requirements. The CRA completes the report by accessing CTEP-AERS via a direct link on the Medidata Rave Expedited Reporting Evaluation form.

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

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

- Study specific documents: Protocols > Documents > Education and Promotion, and
- Expedited Safety Reporting Rules Evaluation user guide: Resources > CTSU Operations Information > User Guides.

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

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

10.3.2 Distribution of Adverse Event Reports

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

10.3.3 Expedited Reporting Guidelines

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

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

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

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

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)

NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** SAEs, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64).

An AE is considered serious if it results in **ANY** of the following outcomes:

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

ALL SAEs that meet the above criteria **MUST** be immediately reported to the NCI via CTEP-AERS within the timeframes detailed in the table below.

Grade 1-2 Timeframes	Grade 3-5 Timeframes
24-Hour notification, 10 Calendar Days	24-Hour notification, 5 Calendar Days

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

Expedited AE reporting timeframes are defined as:

- "24-Hour notification, 5 Calendar Days" - The SAE must initially be reported via CTEP-AERS within 24 hours of learning of the SAE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
- "24-Hour notification, 10 Calendar Days" - The SAE must initially be reported via CTEP-AERS within 24 hours of learning of the SAE, followed by a complete expedited report within 10 calendar days of the initial 24-hour report.

[1]SAEs that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:
Expedited 24-Hour notifications are required for all SAEs followed by a complete report

- Within 5 calendar days for Grade 3-5 SAEs
- Within 10 calendar days for Grade 1-2 SAEs

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

Effective Date: August 30, 2024

10.3.4 Additional Protocol-Specific Expedited Adverse Event Reporting Exclusions

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

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

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

10.4 Routine Adverse Event Reporting

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

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

10.5 Pregnancy

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

10.6 Secondary Malignancy

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

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

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

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

10.7 Second Malignancy

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

11 STUDY CALENDAR

	Pre-Study (21 days)	Cycle 1											Cycle 2- 6										Maintenance Therapy Cycles 7-30	After 1 year	Relapse	End of Study / Progression	Post-Treatment Follow-Up[b]
		Baseline	D1 (+/- 3 days)	D 2	D 3	D 4	D 5	D 6	D 7	D8	D14	D 28	D1 (+/- 3 days)	D 2	D 3	D 4	D 5	D 6	D 7	D 8	D14	D21	D28	Days 1-28 (+/- 3 days)				
Azacitidine[C]			X	X	X	X	X	X	X				X	X	X	X	X	X	X					X[s]				
Venetoclax[d]			X ----------X										X----------X											X				
Pembrolizumab (MK-3475)[e]										X Q3 wks			Q3 wks from C1D8											Q3 weeks				
Informed consent	X																											
Medical history	X																											
EKG	X[f]																											
ECHO or MUGA	X																											
Vital signs and physical exam	X		X									X[x]																X
Height	X																											

	Pre-Study (21 days)	Cycle 1											Cycle 2- 6											Maintenance Therapy Cycles 7-30	After 1 year	Relapse	End of Study / Progression	Post-Treatment Follow-Up[b]
		Baseline	D1 (+/- 3 days)	D 2	D 3	D 4	D 5	D 6	D 7	D8	D14	D 28	D1 (+/- 3 days)	D 2	D 3	D 4	D 5	D 6	D 7	D 8	D14	D21	D28	Days 1-28 (+/- 3 days)				
Pregnancy Test[g]	X																											
Weight	X												X															
Concurrent Medications		X----------																						----------X				
Performance status	X												X[h]															
Comprehensive Serum Chemistry Panel[i, k]	X		X	X	X	X						X[x]	X											X				X
CBC with Differential[j, k]	X		X	X	X	X						X[x]	X											X				X
Pregnancy Test – Urine or Serum β-HCG[i]	X																											
Urinalysis	X		X									X[x]																X
TSH; T3, T4[k]	X									X		X																
Amylase and lipase[f]	X																											

	Pre-Study (21 days)	Cycle 1											Cycle 2- 6											Maintenance Therapy Cycles 7-30	After 1 year	Relapse	End of Study / Progression	Post-Treatment Follow-Up[b]
		Baseline	D1 (+/- 3 days)	D 2	D 3	D 4	D 5	D 6	D 7	D8	D14	D 28	D1 (+/- 3 days)	D 2	D 3	D 4	D 5	D 6	D 7	D 8	D14	D21	D28	Days 1-28 (+/- 3 days)				
Bone marrow aspiration (FTL3 mutations, karyotype)		X																										
Bone marrow aspiration in EDTA for MRD[t,w]		X										X[l]											X[l,m,x]	X[n,o]	X		X	
Bone marrow aspiration in EDTA[w]		X																					X[p,x]		X	X	X	
Blood in EDTA[w]		X										X[l]											X[l,m,x]	X[n,]	X		X	
Blood in sodium heparin[w]		X																					X[p,x]		X	X	X	
Blood in Streck tube [w]		X										X[l]											X[x]	X	X		X	

	Pre-Study (21 days)	Cycle 1											Cycle 2- 6											Maintenance Therapy Cycles 7-30	After 1 year	Relapse	End of Study / Progression	Post-Treatment Follow-Up[b]
		Baseline	D1 (+/- 3 days)	D 2	D 3	D 4	D 5	D 6	D 7	D8	D14	D 28	D1 (+/- 3 days)	D 2	D 3	D 4	D 5	D 6	D 7	D 8	D14	D21	D28	Days 1-28 (+/- 3 days)				
Bone marrow core biopsy[w]		X[o]																					X[o,p,x]		X[o]	X[o]	X	
Bone marrow biopsy as part of standard of care		X										X[r, 1]											X[r,x]					
Skin Punch Biopsy[w]		X																										
Stool,[w]		X										X(+/- 3 days)																
Adverse Events			X---------- X																										

	Pre-Study (21 days)	Cycle 1											Cycle 2- 6										Maintenance Therapy Cycles 7-30	After 1 year	Relapse	End of Study / Progression	Post-Treatment Follow-Up[b]
		Baseline	D1 (+/- 3 days)	D 2	D 3	D 4	D 5	D 6	D 7	D8	D14	D 28	D1 (+/- 3 days)	D 2	D 3	D 4	D 5	D 6	D 7	D 8	D14	D21	D28	Days 1-28 (+/- 3 days)				
Survival Status[q]																											X

a If the patient does not have a CR/CRi before starting this part of the study, the patient should go off study.
b All participants should be followed for survival to 3 years after end of treatment. For patients who achieve undetectable MRD during study treatment, central MRD assessments should continue in addition to general clinical follow.
c Administered at 75 mg/mL^2 IV over 10-40 min daily or SubQ. Treatment cycle begins on Day 1 of azacitidine administration. Days 5-2-2 schedule allowed if weekend administration not available. Allow at least 1 hour after Pembrolizumab completion when given on same day.
d Administered daily at 400 mg orally. Can be interrupted for 14 days to allow for morphologic leukemia and count recovery. From cycle 2+, should be given from either Days 1-21 or Days 1-28 depending on count recovery.
e Only for patients randomized to the intervention arm at 200 mg. Started on Cycle 1 Day 8, then Q3W (+/- 3 days). During the maintenance phase, MK-3457 is administered Q3W (+/- 3 days) for up to 2 years for a maximum of 36 doses.
f As clinically indicated after Pre-study assessment.
g For women of childbearing potential, a serum or urine pregnancy test must be conducted within 72 hours prior to first dose of study treatment on both treatment arms. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.
h Note: Performance status evaluations are based on a 4-week cycle. At minimum, performance status should be evaluated at the beginning of every cycle.
i Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium, uric acid.
j CBCs (with differential and platelet count), which includes WBC, ANC, platelets, Hgb, and Hct required for protocol therapy must be done <24 hours prior to the treatment cycle.
k CBC, CMP, TSH should be performed at baseline and <72 hours prior to each dose of MK-3475 (pembrolizumab) for those on Intervention arm. Additional T3 and T4 tests should be performed as clinically indicated, if TSH is abnormal, or if symptoms are suggestive of thyroid dysfunction.
l To be collected ideally between Day 21 and 28, and as close to day 21 as possible. However, if this was not done for any reason at this time, it must be collected before starting Cycle 2. Bone marrow exam must be done between day 21 and day 42 of cycle 1 even if there is no count recovery with the exact timing of evaluation up to investigator, as long as bone marrow exam is done prior to starting cycle 2.
m To be collected after 2nd, 4th, and 6th cycles.
n To be collected every 3 months during maintenance phase.
o Optional.
p Collected after 4th and 6th cycles
q Information will be collected via questionnaire conducted with a phone call.
r Standard of care bone marrow biopsy will be collected after 1st, 2nd, 4th, and 6th cycles
s Azacitidine will be administered on Days 1-7 or days 5-2-2 (to avoid weekend administration).
t. Bone marrow aspirate is required to be submitted for central MRD testing as outlined in Section 5. Submit to Brent Wood Lab, Children's Hospital, Los Angeles. Results will be forwarded to the submitting institution within 1-2 business days of receipt of the bone marrow aspirate samples. Local bone marrow biopsy (aspirate) must be performed at the indicated time points and should follow the institutional standard of care.
w. Refer to Section 5.1 Summary Table for Specimen Collection
x. +/- 3 days

12 MEASUREMENT OF EFFECT

Responses in AML patients will be assessed using ELN 2017 response criteria (Dohner *et al.,* 2017) *(*Appendix D*)* given shortcomings of the International Working Group 2003 criteria in the era of MRD assessment (Cheson *et al.,* 2003; Bloomsfield *et al.,* 2018).

12.1 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with azacitidine + venetoclax with or without pembrolizumab (MK-3475).

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease reevaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of Cycle 1 will also be considered evaluable). To account for the expected drop-out that might occur during induction before primary end point assessment, we would add another 20% patients to enroll in each arm, for a total planned enrollment of 76 patients. All patients starting induction therapy should be included in the primary analysis with drop-outs considered as not being MRD- for the analysis of rates

12.2 Hematologic Response

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

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

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

12.2.4 **CR with partial recovery count (CRh):** All CR criteria with residual neutropenia > 0.5 x 10^9/L [1000/μL]) and thrombocytopenia (>50 × 10^9/L [100,000/μL] (DiNardo *et al*., 2018b).

12.2.5. **Hematologic improvement:** Erythroid response (E) (pretreatment Hgb <11 g/dL) Hgb increase by >/=1.5 g/dL, Platelet response (P) (pretreatment platelets <100 x10^9/L) Absolute increase of >/=30 x 10^9/L for patients starting with > 20 x 10^9/L platelets Increase from < 20 x 10^9/L to > 20 x 10^9/L and by at least 100%

12.2.6. **Morphologic leukemia-free state (MLFS):** Bone marrow blasts <5%; absence of blasts with Auer rods; absence of extramedullary disease; no hematologic recovery required.

(Marrow should not merely be "aplastic"; at least 200 cells should be enumerated or cellularity should be at least 10%)

12.2.7. **Partial remission (PR)** All hematologic criteria of CR; decrease of bone marrow blast percentage to 5% to 25%; and decrease of pretreatment bone marrow blast percentage by at least 50%

Peripheral Blood Counts:

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

Bone Marrow Aspirate and Biopsy:

- Cellularity of bone marrow biopsy must be >20% with maturation of all cell lines.
- <5% blasts
- Auer rods must not be detectable.

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

Note: stable disease would not count as an adequate response in terms of response assessment for study purposes. However, some patients with stable disease can also achieve hematologic improvements such as transfusion independence without reduction in blasts, and some patients on immunotherapy arm might need longer time than 6 months to respond or could have more stable disease that translates into longer survival in this randomized study. Furthermore, there are very few options available for most patients after azacitidine-venetoclax and therefore it imperative to ensure patients can have the opportunity to achieve maximal benefit from this therapy. We therefore will allow the treating investigator to keep patients with stable disease at their discretion if they judge the patient to be deriving clinical benefit on study, that is defined by transfusion independence (defined as at least 8 consecutive weeks without transfusions of either red blood cells or platelets for patients who were receiving transfusions prior to or during therapy) or by hematologic improvement criteria as listed in section 12.2.5 of the protocol.

12.2.8 MRD Response

Refer to section 9.1.1

12.3 Other Response Parameters

- EFS or RFS will be calculated as the time from initial treatment to either disease relapse or death. Duration of response of remission duration will be calculated as the time from first CR to the date of the first documented relapse or death, whichever occurs first. OS will be calculated from time from initial treatment to death.

13 STUDY OVERSIGHT AND DATA REPORTING / REGULATORY REQUIREMENTS

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

13.1. Study Oversight

This protocol is monitored at several levels, as described in this section. The Protocol Principal Investigator is responsible for monitoring the conduct and progress of the clinical trial, including the ongoing review of accrual, patient-specific clinical and laboratory data, and routine and serious adverse events; reporting of expedited adverse events; and accumulation of reported adverse events from other trials testing the same drug(s). The Protocol Principal Investigator and statistician have access to the data at all times through the CTMS web-based reporting portal.

During the Phase 2 portion of the study, the Protocol Principal Investigator will have, at a minimum, quarterly conference calls with the Study Investigators and the CTEP Medical Officer(s) to review accrual, progress, and pharmacovigilance.

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

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

13.2. Data Reporting

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

- Site staff will need to be registered with CTEP and have a valid and active CTEP-IAM account, and
- Assigned one of the following Rave roles on the relevant Lead Protocol Organization (LPO) or Participating Organization roster at the enrolling site: Rave CRA, Rave Read Only, Rave CRA (LabAdmin), Rave SLA, or Rave Investigator. Refer to https://ctep.cancer.gov/investigatorResources/default.htm for registration types and documentation required.
    - To hold Rave CRA or Rave CRA (Lab Admin) role, site staff must hold a minimum of an AP registration type,
    - To hold Rave Investigator role, the individual must be registered as an NPIVR or IVR, and

- To hold Rave Read Only role, site staff must hold an Associates (A) registration type.

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

Upon initial site registration approval for the study in Regulatory Support System (RSS), all persons with Rave roles assigned on the appropriate roster will be sent a study invitation e-mail from iMedidata. To accept the invitation, site staff must log in to the Select Login (https://login.imedidata.com/selectlogin) using their CTEP-IAM username and password, and click on the *accept* link in the upper right-corner of the iMedidata page. Site staff will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings), and can be accessed by clicking on the link in the upper right pane of the iMedidata screen. If an eLearning is required and has not yet been taken, the link to the eLearning will appear under the study name in iMedidata instead of the *Rave EDC* link; once the successful completion of the eLearning has been recorded, access to the study in Rave will be granted, and a *Rave EDC* link will display under the study name.

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

13.2.1. Method

.
This study will be monitored by the Clinical Trials Monitoring Service (CTMS). Data will be submitted to CTMS at least once every two weeks via Medidata Rave (or other modality if approved by CTEP). Information on CTMS reporting is available at: http://www.theradex.com/clinicalTechnologies/?National-Cancer-Institute-NCI-11. On-site audits will be conducted on an 18-36 month basis as part of routine cancer center site visits. More frequent audits may be conducted if warranted by accrual or due to concerns regarding data quality or timely submission. For CTMS monitored studies, after users have activated their accounts, please contact the Theradex Help Desk at 609-619-7862 or by email at CTMSSupport@theradex.com for additional support with Rave and completion of CRFs.

13.2.2. Responsibility for Data Submission

For ETCTN trials, it is the responsibility of the PI(s) at the site to ensure that all investigators at the ETCTN Sites understand the procedures for data submission for each ETCTN protocol and

that protocol specified data are submitted accurately and in a timely manner to the CTMS via the electronic data capture system, Medidata Rave.

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

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

An End of Study CRF is to be completed by the PI, and is to include a summary of study endpoints not otherwise captured in the database, such as (for phase 1 trials) the recommended phase 2 dose (RP2D) and a description of any dose-limiting toxicities (DLTs). CTMS will utilize a core set of eCRFs that are Cancer Data Standards Registry and Repository (caDSR) compliant (http://cbiit.nci.nih.gov/ncip/biomedical-informatics-resources/interoperability-and-semantics/metadata-and-models). Customized eCRFs will be included when appropriate to meet unique study requirements. The PI is encouraged to review the eCRFs, working closely with CTMS to ensure prospectively that all required items are appropriately captured in the eCRFs prior to study activation. CTMS will prepare the eCRFs with built-in edit checks to the extent possible to promote data integrity.

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

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

13.3. Data Quality Portal

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

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

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

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

Note: Some Rave protocols may not have delinquent form details or reports specified on the DQP. A protocol must have the Calendar functionality implemented in Rave by the Lead Protocol Organization (LPO) for delinquent form details and reports to be available on the DQP. Site staff should contact the LPO Data Manager for their protocol regarding questions about Rave Calendaring functionality.

13.4. Collaborative Agreements Language

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

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

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

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

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

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

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

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

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

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

Email: ncicteppubs@mail.nih.gov

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

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APPENDIX A PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale	
Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
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).
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.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

APPENDIX B FORMULA TO ESTIMATE RENAL FUNCTION USING SERUM CREATININE

Formulas to estimate renal function using serum creatinine provided by the NCI's Investigational Drug Steering Committee (IDSC) Pharmacological Task Force in table below.

1. Estimated glomerular filtration rate (eGFR) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) (Levey *et al.*, 2009).

Formulae:

Race and Sex	Serum Creatinine (SCr), *µmol/L (mg/dL)*	Equation
Black		
Male	≤80 (≤0.9)	$GFR = 163 \times (SCr/0.9)^{-0.411} \times (0.993)^{Age}$
	>80 (>0.9)	$GFR = 163 \times (SCr/0.9)^{-1.209} \times (0.993)^{Age}$
White or other		
Male	≤80 (≤0.9)	$GFR = 141 \times (SCr/0.9)^{-0.411} \times (0.993)^{Age}$
	>80 (>0.9)	$GFR = 141 \times (SCr/0.9)^{-1.209} \times (0.993)^{Age}$

SCr in mg/dL; Output is in mL/min/1.73 m^2 and needs no further conversions.

2. eGFR using the Modification of Diet in Renal Disease (MDRD) Study (Levey *et al*., 2006).

175 x $SCr^{-1.154} \times age^{-0.203} \times 0.742$ (if female) × 1.212 (if black)
Output is in mL/min/1.73 m^2 and needs no further conversions.

3. Estimated creatinine clearance (ClCr) by the Cockcroft-Gault (C-G) equation (Cockcroft and Gault, 1976).

$$CLcr\ (mL/min) = \frac{[140 - age\ (years)] \times weight\ (kg)}{72 \times serum\ creatinine\ (mg\ /\ dL)} \{\times 0.85\ for\ female\ patients\}$$

Followed by conversion to a value normalized to 1.73 m^2 with the patient's body surface area (BSA).

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APPENDIX C PATIENT CLINICAL TRIAL WALLET CARD

NIH NATIONAL CANCER INSTITUTE

CLINICAL TRIAL WALLET CARD

Show this card to all of your healthcare providers and keep it with you in case you go to the emergency room.

Patient Name:

Diagnosis:

Study Doctor:

Study Doctor Phone #:

NCI Trial #: 10334

Study Drug(S): MK-3475 (pembrolizumab), venetoclax, and azacitidine

For more information: 1-800-4-CANCER
cancer.gov | clinicaltrials.gov

APPENDIX D RESPONSE CRITERIA (PER ELN 2017 RECOMMENDATIONS)

Response criteria in AML
Response

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

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

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

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

Partial remission (PR) All hematologic criteria of CR; decrease of bone marrow blast percentage to 5% to 25%; and decrease of pretreatment bone marrow blast percentage by at least 50%

Treatment failure
Primary refractory disease: No CR or CRi after 2 courses of intensive induction treatment; excluding patients with death in aplasia or death due to indeterminate cause

Death in aplasia: Deaths occurring ≥7 d following completion of initial treatment while cytopenic; with an aplastic or hypoplastic bone marrow obtained within 7 d of death, without evidence of persistent leukemia

Death from indeterminate cause: Deaths occurring before completion of therapy, or <7 d following its completion; or deaths occurring ≥7 d following completion of initial therapy with no blasts in the blood, but no bone marrow examination available

Response criteria for clinical trials

Stable disease: Absence of CRMRD−, CR, CRi, PR, MLFS; and criteria for PD not met Period of stable disease should last at least 3 months

Progressive disease (PD): Evidence for an increase in bone marrow blast percentage and/or increase of absolute blast counts in the blood
• >50% increase in marrow blasts over baseline (a minimum 15% point increase is required in cases with <30% blasts at baseline; or persistent marrow blast percentage of >70% over at least 3

mo; without at least a 100% improvement in ANC to an absolute level (>0.5 × 10^9/L [500/μL], and/or platelet count to >50 × 10^9/L [50 000/μL] non-transfused)
• >50% increase in peripheral blasts (WBC × % blasts) to >25 × 10^9/L (>25 000/μL) (in the absence of differentiation syndrome)
• New extramedullary disease Some protocols may allow transient addition of hydroxyurea to lower blast counts
"Progressive disease" is usually accompanied by a decline in ANC and platelets and increased transfusion requirement and decline in performance status or increase in symptoms

Relapse

Hematologic relapse (after CRMRD−, CR, CRi) Bone marrow blasts ≥5%; or reappearance of blasts in the blood; or development of extramedullary disease

Molecular relapse (after CRMRD−) If studied pretreatment, reoccurrence of MRD as assessed by RT-qPCR or by MFC

APPENDIX E PATIENT DIARY

CTEP-assigned Protocol # 10334
Local Protocol # _____

PATIENT'S MEDICATION DIARY – Venetoclax – CYCLE 1

Today's date _____ **Agent**_____

Patient Name_____(*initials acceptable)* **Patient Study ID**_____

INSTRUCTIONS TO THE PATIENT:

1. You will take your dose of venetoclax every single day at the same time for the first 28 days of the first cycle (Days 1-28). On Day 1 you will take 100mg daily dose. On Day 2 you will take 200 mg daily dose. On Days 3-28 you will take 400 mg daily dose.
2. **Take venetoclax with a meal**.. You should swallow the tablets whole. **Do not chew**.
3. If you have any comments or notice any side effects, please record them in the Comments column.
4. Please return the forms to your physician when you go for your next appointment.

Day	Date	What time was dose taken?	# of 100mg tablets taken	Comments
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		(-)		

Physician's Office will complete this section:

1. Date patient started protocol treatment _____
2. Date patient was removed from study _____
3. Patient's planned total daily dose _____

4. Total number of pills taken this month (each size) _____
5. Physician/Nurse/Data Manager's Signature _____

Patient's signature: _____

CTEP-assigned Protocol # 10334
Local Protocol # _____

PATIENT'S MEDICATION DIARY – Venetoclax – CYCLE 2+

Today's date _____ **Agent**_____

Patient Name_____(*initials acceptable)* **Patient Study ID**_____

INSTRUCTIONS TO THE PATIENT:

1. Complete one form for each month.
2. You will take your dose of venetoclax on Days 1-28 or Days 1-21 depending on your blood count of Cycle 2 onwards. .
3. **Take venetoclax with a meal**.. You should swallow the tablets whole. **Do not chew**.
4. If you have any comments or notice any side effects, please record them in the Comments column.
5. Please return the forms to your physician when you go for your next appointment.

Day	Date	What time was dose taken?	# of100mg tablets taken			Comments
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		(-)				

Physician's Office will complete this section:

1. Date patient started protocol treatment _____
2. Date patient was removed from study _____
3. Patient's planned total daily dose

4. Total number of pills taken this month (each size) _____
5. Physician/Nurse/Data Manager's Signature _____

Patient's signature: _____

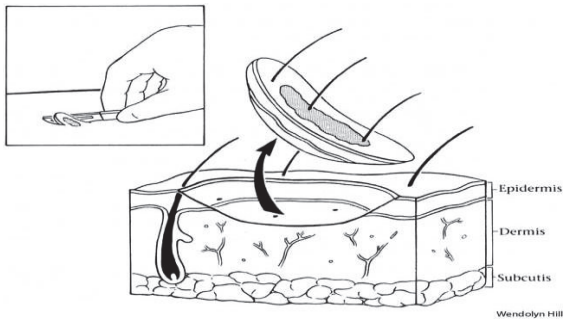
APPENDIX F CUTTING AND EMBEDDING SKIN

Skin Specimens - General Considerations for FFPE tissue block preparations: It is crucial to maintain vertical orientation at all times in the sections.

A. Ink can be used to highlight areas of the specimen, including how to orient it when embedding (e.g., inked dermal/subcutaneous margins). Black or blue inks are preferred.
B. Diagrams should be used for any difficult or complicated biospecimens.

Skin Shave Biopsies

A. Principle: Ink the dermal/subcutaneous margins of all skin specimens.



B. Processing the Specimen
    1. The specimen type (shave) dimensions (including depth and surface appearance) are described. The specimen is usually oval and relatively flat. The edges may curl secondary to retraction of the dermis. Specimens larger than 4 mm in diameter should be bisected, larger than 6 mm in diameter should be trisected, larger than 8 mm should be quadrisected or serially sectioned, and end sections submitted in a separate cassette. The vertical orientations should be maintained by making cuts perpendicular to the surface at 2-3 mm intervals.

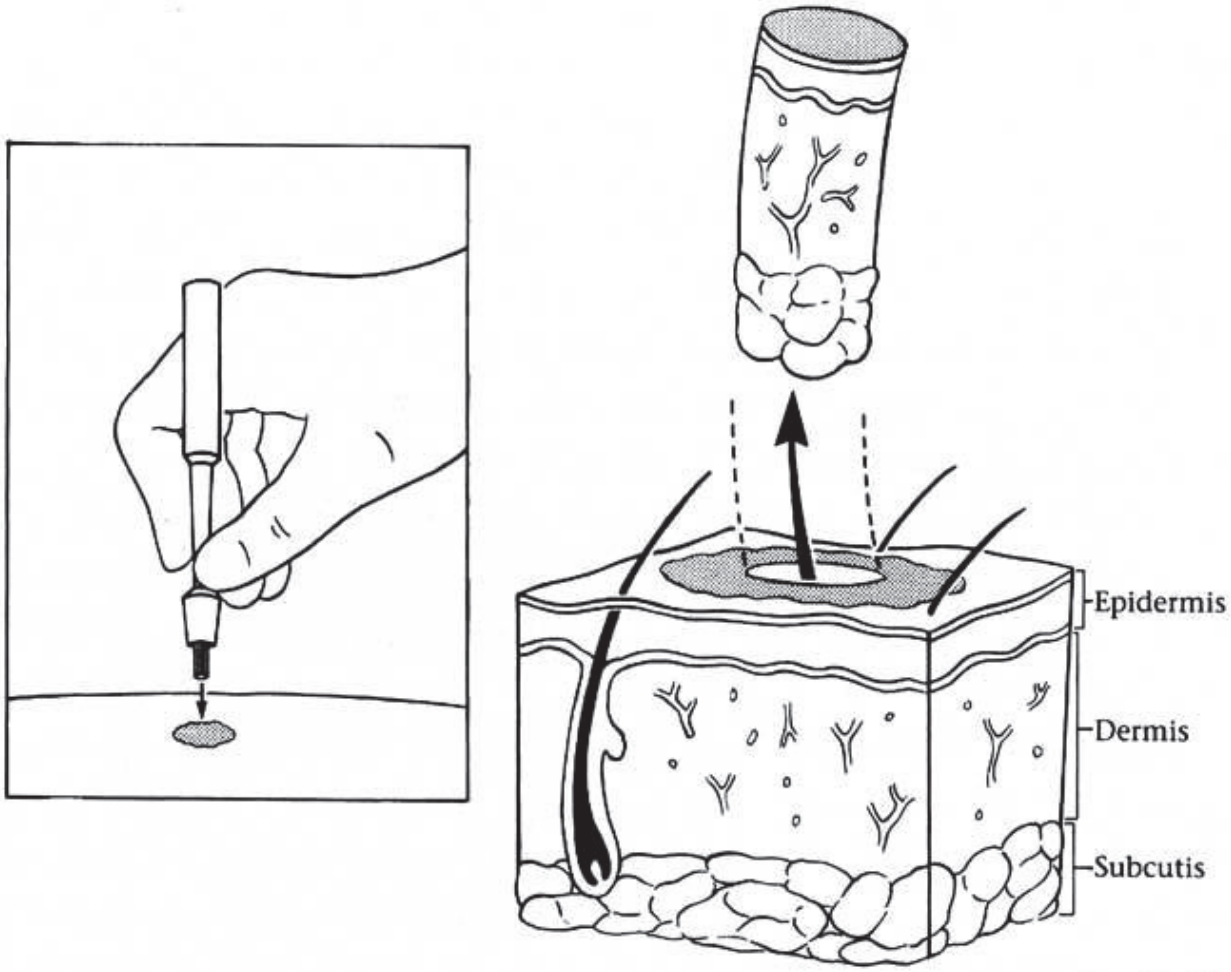
    2. For small shaves < 4mm, the intact specimen should be placed on edge (aka: cut side down) in a cassette.

    3. For larger shaves > 4mm and < 8 mm, the sections should be placed in a single cassette on edge.

    4. For shaves > 8 mm, the sections should be placed in 2 or more cassettes with the ends in 1 cassette.

Skin Punch Biopsies

A. Principle: Punch biopsies are performed to completely excise small lesions or to sample large lesions. Punches can range from 2-8 mm in diameter.



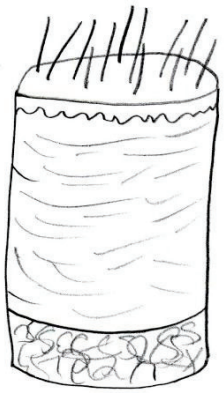
B. Processing the specimen

1. The type of specimen (punch biopsy) is described including diameter, depth, and skin color. Lesions are described including size, type (macular, papular, vesicular, plaque), borders (well-circumscribed, irregular), color, shape (verrucous, lobulated, bosselated).

2. Apply ink to the cut surfaces of all punch biopsies.

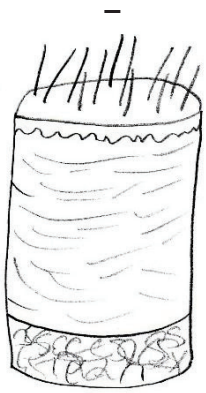
3. See below instructions for specific sectioning details

Punch Biopsy < 4mm in diameter



Lay specimen on its side intact in a cassette

Punch Biopsy > 4mm in diameter

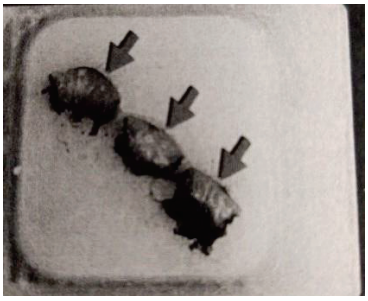


Bisect punch lengthwise and place both halves with cut surface from middle of specimen face down in cassette.

Embedding – Summary

Skin shave biopsies
Orientation: Embed the cut surface of the skin sliver to be positioned perpendicularly so that all layers will be displayed. Shave biopsies often will resemble “crescent” shapes when embedded. Use the epidermal surface to assist orientation.

When a specimen is inked, all inked edges should face the same direction in the block face.



Skin punch biopsies - not bisected:
Orientation: Embed with the long side with epidermal disc on edge to show epidermis, dermis and subcutaneous fat layer in cross-section. The skin pieces should be placed at a slight angle with the epidermis facing all in the same direction (multiple pieces). The specimen and the epidermis must be pressed to lie in the same plane. The entire epidermal edge must be present on the completed slides.

Skin punch biopsies - bisected
Orientation: Embed cut surfaces down, long side with epidermal disc on edge to show epidermis, dermis and subcutaneous fat layer in cross-section.

Notes:

1. Many times, special instructions for embedding are noted, especially if the histology laboratory has a specific SOP for embedding skin lesions.
2. Identify the tissue in the cassette. Use submitted descriptions regarding the gross appearance of the biopsied specimen (e.g., features of the lesion may include color, ulceration, polypoid configuration).
3. Check if the specimen was inked and if there is a description of the reason for inking (e.g., identify the cut surface or the dermal/subcutaneous margin). If the specimen is inked be sure to follow embedding instructions (e.g., inked side down).
4. Choose a mold that is of adequate size for the dimensions of the tissue.
5. If you re embedding very small samples, use a magnifying glass to arrange them on edge.
6. If the edges of the skin specimen curl due to retraction (commonly seen with shave biopsies), uncurl the tissue gently with forceps and warm them on the embedding center before embedding on edge. Skin shave biopsies should be straightened before paraffin hardens completely.

Slide preparation - The FFPE tissue block will be trimmed or "faced" until the full outline of the tissue is visible. Cut at 3-4 microns.

For layered skins (e.g., skin shave biopsies >4 mm), the epidermis should be facing toward the embedding unit, cassette number facing to the right. The skin pieces should be placed at a slight angle with the epidermis facing all in the same direction (multiple pieces). The specimen and the epidermis must be pressed to lie in the same plane. The entire epidermal edge must be present on the completed slides.

APPENDIX G BONE MARROW CORE AND SKIN PUNCH BIOPSY SAMPLES

- Tissue ***must be fixed*** in neutral-buffered formalin (no acid-based products).
- ***For collection sites shipping samples in Ethanol,*** formalin fixed tissue will be transferred to 70% ethanol at room temperature for **up to 72** hours before processing (Steps 3 to 13, Table 1) is completed at the Biorepository.
- The tissue will be processed on an ***automated tissue processor*** following Steps 3 to 12 ***as suggested*** in Table 1 so long ***as total time from ethanol to embedding (in gray) exceeds 4 hours.***
- Do ***not*** use a microwave processor.
- The tissue will be embedded in paraffin (Step 13, Table 1).

Table 1. **Main stages of tissue processing.** Steps 3-12 performed in an automated tissue-processor (no microwave processors).

Step/Process	Solution	Time
1. Fixation	10% buffered formalin	12-24 hours
2.Dehydration	70% Ethanol	30 minutes or up to 72 hours
3.Dehydration	95% Ethanol	30 minutes
4.Dehydration	95% Ethanol	30 minutes
5.Dehydration	100% Ethanol	30 minutes
6.Dehydration	100% Ethanol	30 minutes
7.Dehydration	100% Ethanol	30 minutes
8.Clearing	Xylene	30 minutes
9.Clearing	Xylene	30 minutes
10.Infiltration	Paraffin Wax	30 minutes
11.Infiltration	Paraffin Wax	30 minutes
12.Infiltration	Paraffin Wax	30 minutes
13.Blocking Out	Paraffin Wax	n/a