SUMMARY OF CHANGES – Protocol

For Protocol Revision 20 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 1 (BLAST MRD AML-1): A Randomized Phase 2 Study of the Anti-PD-1 Antibody Pembrolizumab in Combination with Conventional Intensive Chemotherapy as Frontline Therapy in Patients with Acute Myeloid Leukemia

NCI Protocol #: 10300
Local Protocol #: 2000028858

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I. Update by Principal Investigator

Section	Comments
All	Updated Version Date in Header
Title Page	Revised Version# / Version Date
5.8	Updates to the **CIMAC biomarker plan**: Added the following to the Biomarker Plan table: • Corrected "end of treatment" to "relapse" for blood-based CIMAC assays • Added relapse timepoint to WES in bone marrow aspirate • Added TCRseq in peripheral blood • Updated TCRseq lab as Adaptive Biotechnologies working with MDACC CIMAC • Upated MDACC CIMAC Lab Co-PI's.
6.0	Updated shipping addresses and lab contacts. Noted EDTA blood can also be used for TCRseq.

NCI Protocol #: 10300

Local Protocol #: 2000028858

ClinicalTrials.gov Identifier: NCT04214249

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 1 (BLAST MRD AML-1): A Randomized Phase 2 Study of the Anti-PD-1 Antibody Pembrolizumab in Combination with Conventional Intensive Chemotherapy as Frontline Therapy in Patients with Acute Myeloid Leukemia

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NCI-Supplied Agent: Pembrolizumab (MK-3475) (NSC # 776864)
Other Agents: Cytarabine (NSC # 63878) and either Idarubicin (NSC # 256439) or Daunorubicin (NSC # 82151)

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Revision 1 / June 20, 2019
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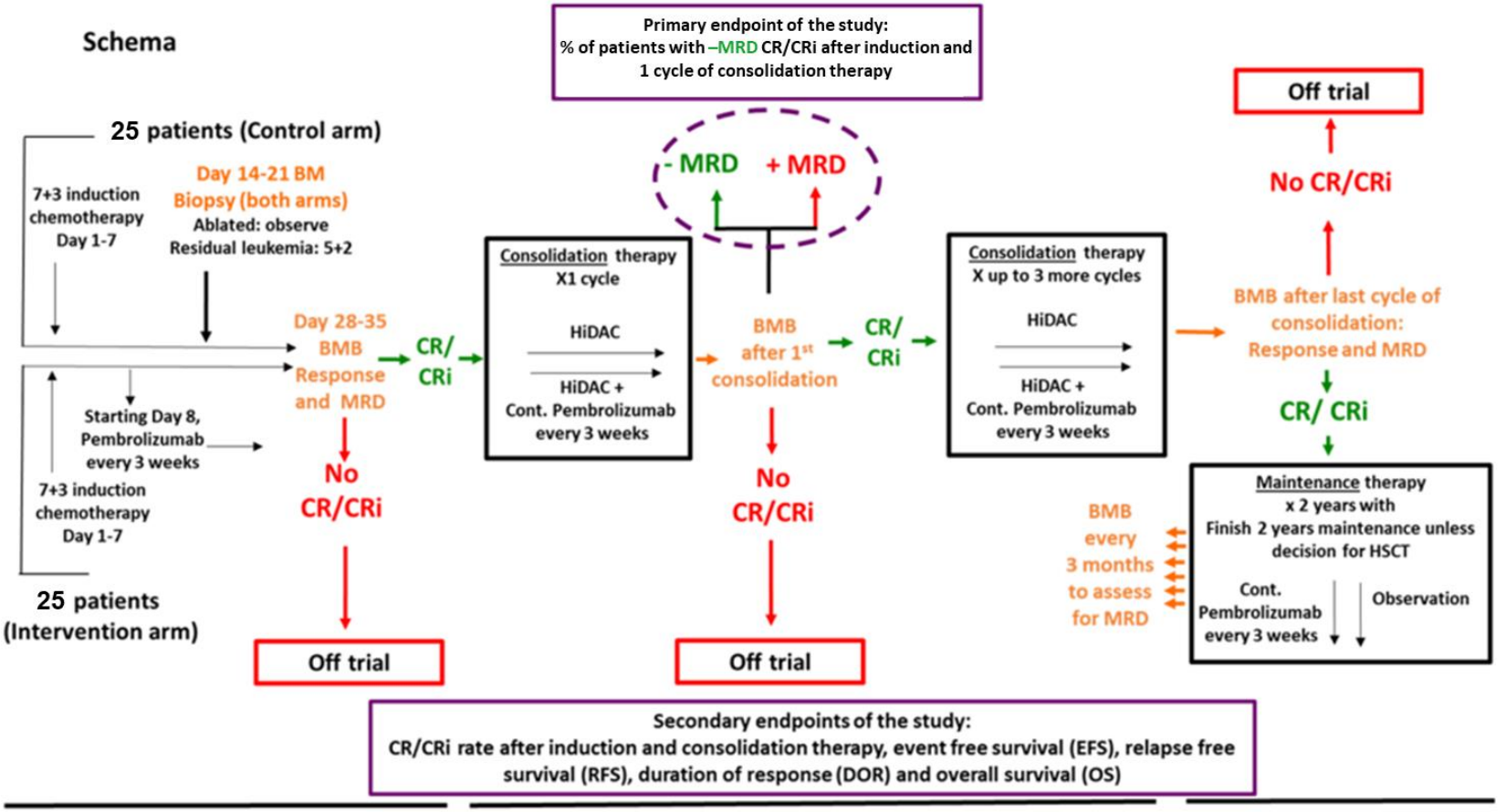
Revision 18 / October 23, 2024
Revision 19 / November 26, 2024
Revision 20 / April 10, 2025

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 To assess the percentage of patients with minimal residual disease (MRD) negative complete remission (CR) (MRD-CR), or with minimal residual disease (MRD) negative complete remission with incomplete recovery (CRi) (MRD-CRi) as measured by flow cytometry at the end of first cycle of consolidation therapy with chemotherapy + Pembrolizumab (MK-3475) and compare between the two study arms.

1.2 Secondary Objectives

1.2.1 Assess the rate of complete remission (CR) / complete remission with incomplete count recovery (CRi) as defined per European Leukemia Net 2017 response criteria at time of count recovery after induction therapy with chemotherapy + Pembrolizumab (MK-3475) (Dohner *et al.*, 2017).

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

1.2.3 Assess the rates of MRD negativity at Day 14, MRD-negative CR at end of induction therapy and MRD negative CR after last consolidation cycle.

1.2.4 Assess event free survival (EFS), measured from randomization to failure to achieve CR/CRi, relapse or death from any cause, and relapse free survival (RFS), calculated as the time from first documentation of CR/CRi to either disease relapse or death from any cause.

1.2.5 Assess the 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), defined as time from randomization to death from any cause.

1.2.6 Assess safety endpoints including proportion of patients who develop severe toxicity as defined in the protocol.

1.3 Exploratory Objectives

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

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

2. BACKGROUND

2.1 Study Disease

2.1.1 Acute myeloid leukemia (AML) and minimal residual disease (MRD)

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).

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 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). Kroenig *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.

2.1.2.3 Early 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 azacytidine (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-1$^+$T$_{reg}$ cell infiltrate in the bone marrow. The ratio of PD-1$^+$CD8$^+$ T-effector cells to PD-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 The role of anthracycline-based chemotherapy in modulating the immune system

Over the last years it has become apparent that chemotherapy does not only cause death of tumor cells by cytostatic effects alone but also by stimulating an immune response directed towards cancer cells by reinstating immune surveillance (Kroemer *et al*., 2013; Zitvogel *et al*., 2013).

Chemotherapy has been demonstrated to augment the immune response against cancer through multiple mechanisms including improved antigen uptake and chemotactic response by macrophages and dendritic cells, improved recognition of neoplastic neoepitopes over major

histocompatibility (MHC) complex class I and T-cell receptors, and increased susceptibility of tumor cells to immune-mediated cytotoxicity (Zitvogel *et al*., 2011; Galluzzi *et al*., 2012). The key in electing an immune response to cancer cells in response to chemotherapy is the induction of an immunogenic cell death (ICD) as opposed to a non-immunogenic cell death (non-ICD) like apoptosis (Kroemer *et al*., 2013). In order to induce ICD, chemotherapeutic agents need to induce pre-apoptotic exposure of calreticulin (CRT) at the cell surface by inducing endoplasmic reticulum (ER) stress (Panaretakis *et al*., 2009), the secretion of ATP during the blebbing phase of apoptosis (Michaud *et al*., 2011), and the cell death–associated release of the non-histone chromatin protein high-mobility group box 1 (HMGB1) (Apetoh *et al*., 2007). CRT release (ecto-CRT) from tumor cells is a potent "eat me" signal leading to an engulfment by macrophages and dendritic cells (Gardai *et al*., 2005). In AML patients, the spontaneous exposure of CRT by leukemic cells has been shown to predict antitumor T-cell responses and improved patient survival (Wemeau *et al*., 2010). Importantly, CRT only leads to an immunogenic cell death if it is not set off by the expression of "don't eat me" signals like CD47 (Vonderheide, 2015). Importantly, the 'eat me' signal ecto-CRT was shown to inversely correlate with the expression of the 'don't eat me' signal CD47 in AML blasts (Wemeau *et al*., 2010). ATP released from tumor cells is a potent "find me" signal (chemotaxis) for dendritic cells and macrophages (Elliott *et al*., 2009). Interestingly, only a small selection of chemotherapeutic agents are able to induce ICD in cancer cells: when cancer cells were exposed to 24 different chemotherapeutic agents, only four agents (three anthracyclines and oxaliplatin) were able to induce ICD while all agents were able to induce apoptosis (Obeid *et al*., 2007). It seems that anthracyclines are particularly potent in inducing ICD, which is important for AML therapy since the backbone of AML 7+3 chemotherapy is anthracyclines (Fucikova *et al*.,2011).

2.2 CTEP IND Agent

2.2.1 Pembrolizumab (MK-3475)

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

MK-3475 (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 "7+3" induction chemotherapy

The most commonly used induction regimen for AML is the so-called "7+3" regimen, which has been

reviewed in detail before (Dohner *et al*., 2010). This regimen combines a seven-day continuous IV infusion of cytarabine (100 or 200 mg/m^2 per day) with a short infusion or bolus of an anthracycline given on days 1 through 3. The most commonly used anthracycline in this regimen is daunorubicin, but idarubicin is sometimes used as well. The combination of "standard dose" cytarabine plus daunorubicin has been the historical standard for remission induction in AML. In this regimen, cytarabine is given by continuous IV infusion for 7 days plus daunorubicin by IV push or short infusion daily for the first three days. Standard dosing for patients less than 60 years old is: Cytarabine 200 mg/m^2 for 7 days plus daunorubicin 60-90 mg/m^2 for 3 days. Idarubicin is administered at 12 mg/m^2/day for 3 days in combination with Cytarabine 200 mg/m^2 for 7 days.

2.3.1.1 Pharmacokinetics

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

Daunorubicin distributes widely into tissues, particularly the liver, kidneys, lung, spleen, and heart; does not distribute into the central nervous system (CNS). Daunorubicin is metabolized primarily hepatically to daunorubicinol (active), then to inactive aglycones, conjugated sulfates, and glucuronides. It is excreted in feces (40%) and urine (~25% as unchanged drug and metabolites). Initial daunorubicin half-life elimination is 45 minutes; terminal cytarabine half-life elimination is 18.5 hours. Idarubicin is metabolized hepatically to idarubicinol (active metabolite) and excreted primarily by biliary tract and in the urine (8 to 10%). The elimination half-life is 22 hours (range: 4 to 48 hours).

2.3.1.2 Efficacy

Depending on age and patient selection, 70% to 80% of younger adults achieve a CR with these regimens. Most remissions happen after a single course. In the past, daunorubicin had been commonly employed at a dose of 45 mg/m^2; however, randomized studies suggest that higher doses of daunorubicin (*e.g*., 60 to 90 mg/m^2 per day) are more effective and no more toxic than 45 mg/m^2 and that 90 mg/m^2 was no more effective, but more toxic than 60 mg/m^2.

2.3.1.3 Safety data

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

2.4 Rationale

Combining 7+3 induction chemotherapy with immune checkpoint inhibitor therapy could lead to synergy by combining the immunomodulatory abilities of checkpoint inhibition and traditional chemotherapy. Chemotherapy with anthracyclines 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 as discussed earlier (Kroemer *et al*., 2013; Zitvogel *et al*., 2013). 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 induction chemotherapy. Adding the anti PD-1 antibody Pembrolizumab (MK-3475) to 7+3 induction chemotherapy may lead to more effective CTL-mediated destruction of leukemic blasts and thereby results in improved rates of CR without MRD and thereby lower relapse rates.

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 (HiDAC followed by MK-3475 [pembrolizumab]) (Zeidner *et al.*, 2017) and epigenetic therapy (AZA 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 (AZA 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. Importantly, preliminary results from AML trials using anti-PD1 therapy in combination with intensive chemotherapy in the relapsed/refractory setting and high doses of cytarabine/idarubicin in frontline setting and in combination with hypomethylating agents among the patients who proceeded to HSCT after checkpoint inhibitor therapy found no signal for increased risk of GVHD or transplant-related toxicity (Ravandi *et al.*, 2017; Zeidner *et al.*, 2017).

We hypothesize that standard anthracycline based induction chemotherapy in combination with the anti-PD-1 antibody Pembrolizumab (MK-3475) is associated with acceptable toxicity profile and leads to an improvement in the percentage of patients with MRD negative CR. We hypothesize that standard anthracycline induction in combination with the anti-PD-1 antibody, Pembrolizumab (MK-3475), would lead to an increase in the percentage of MRD negative CR from 50% to 75% after induction and one cycle of consolidation therapy.

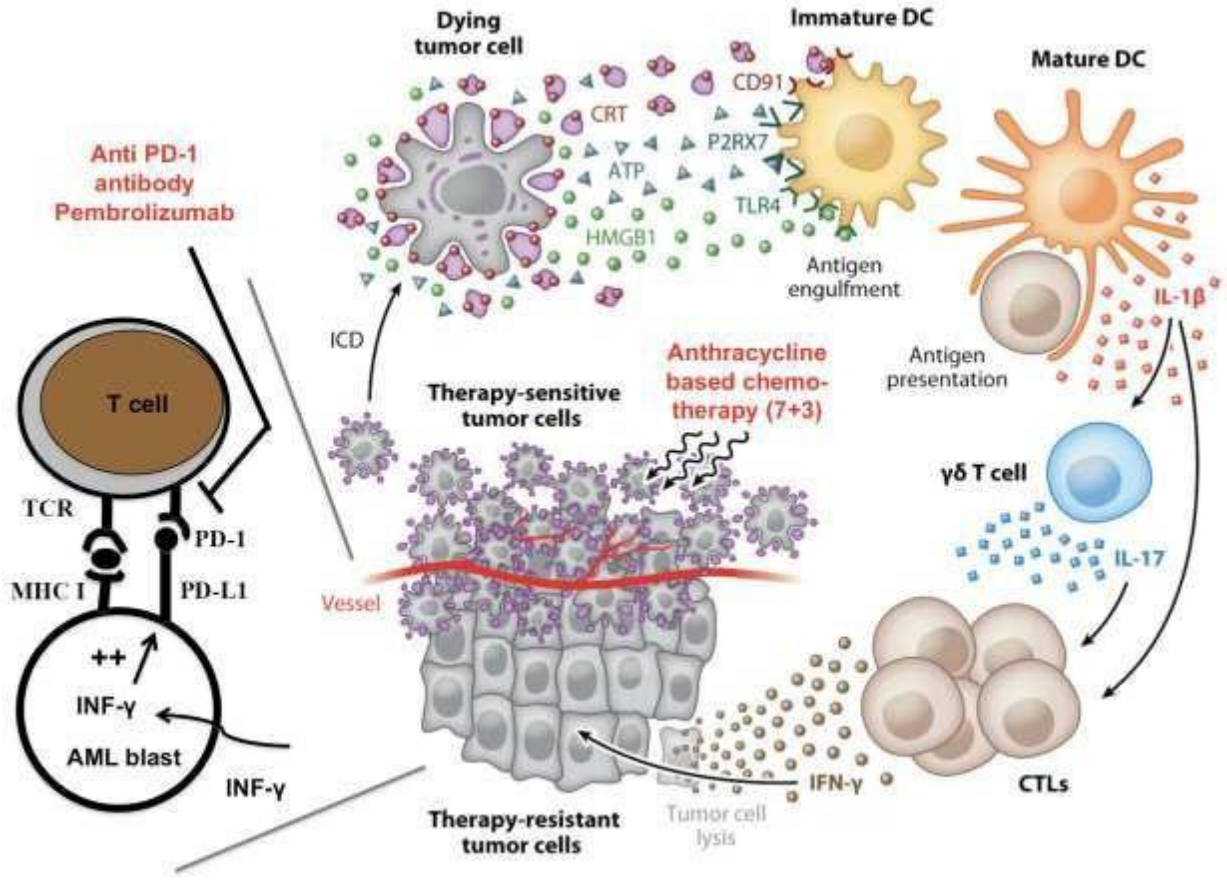


Figure 1. Potential synergistic effect of 7+3 induction chemotherapy and checkpoint inhibition with anti PD-1 antibody Pembrolizumab (MK-3475) in inducing an immune response against AML blasts. Figure modified from Kroemer *et al*., (2013).

2.4.1 Standard of care assessments:

2.4.1.1 Core Binding Factors

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). Unlike the case with the favorable risk AML, intermediate

and 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 use CBF (by karyotype or FISH) as a stratification factor to account for heterogeneity in outcomes based on AML risk status.

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. In any case, results from FISH or karyotype should show if CBF abnormalities are present by at time of randomization as the presence of CBF abnormalities is a required stratification factor.

2.4.1.2 2.4.1.2 *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. Since the current trial will not be incorporating a FLT3 inhibitor as a part of the treatment, we will exclude *FLT3*-mutated AML in this study.

2.5 Correlative Studies Background

2.5.1 Assessment of MRD

2.5.1.1 MRD assessment by MFC as an integral biomarker

The flow cytometric MRD assay is an established and validated assay and is used as the reference method for this comparison. While an integral biomarker in the study, Study-based therapeutic decisions will not be based on the results of the MRD flow assay. Patients with AML will have flow cytometry in the bone marrow (BM) at the time of study entry to determine leukemia- associated immunophenotypic abnormalities and then BM will be evaluated after one cycle of consolidation therapy (primary endpoint).

The central MRD studies will be performed solely in the flow cytometry laboratory located in 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
AMLM1	HLA-DR	CD15	CD33	CD19	CD117	CD13	CD38	CD34	CD71	CD45
AMLM2	HLA-DR	CD64	CD123	CD4	CD14	CD13	CD38	CD34	CD16	CD45
AMLM4	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.

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

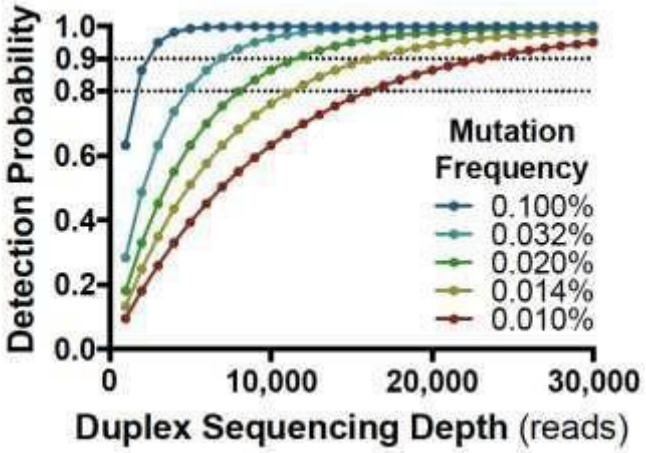
NGS provides a powerful new platform for MRD detection. Using NGS, millions of DNA fragments can be sequenced in parallel and pieced together to determine the sequence of large

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 2, below).

Patients with AML will have DS performed on the bone marrow (BM) and PB at the time of study entry,



at Day 14, at the end of induction therapy (EOI), after one cycle of consolidation therapy, and at the end of consolidation therapy (EOC), and at end of maintenance therapy/observation period for patients who do not progress. For patients who are willing to have BM done every 3 months during follow up, DS in BM and PB could be assessed during maintenance therapy/observation period.

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).

2.5.2.1 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 (alloHSCT) 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 laboratory researchers conducting this assay will be blinded to MFC results.

2.5.2.2 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 8mL 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.

2.5.3 Assessment of immune cell subsets and its correlation with the response to combine chemotherapy and anti-PD-1 directed therapy

Currently, there is no single biomarker to predict the response to anti-PD-1 Ab therapy especially in myeloid leukemia. Two recent landmark studies suggest that the predicted response of anti- PD-1 is associated with PD-L1 expression in tumor-infiltrating immune cells (Herbst *et al*., 2014; Powles *et al*., 2014). These studies have led us to test the question about the expression of PD-L1 on blasts from human AML BM and blood and its correlation with the response to combined anti-PD-1/chemotherapy in AML.

The most reliable biomarker to predict anti-PD-1 Ab therapy includes tumor-infiltrating lymphocytes (TILs). However, further evaluation of TILs subpopulations and their interaction with other TME components will be necessary to identify other response-predictive factors. Compared to a murine model for linear T-cell differentiation, we have had to rely on cross-sectional assessment to define memory T-cell subsets. More than two markers (CD45RA, CCR7) enabled us to delineate memory T-cell compartments (Sallusto *et al*., 1999). For instance, we simply divide T-cells into four subsets: naïve ($CD45RA^+CCR7^+$), central memory ($CD45RA^-CCR7^+$), effector memory ($CD45RA^-CCR7^-$), and late effector ($CD45RA^+CCR7^-$). The ratio of TCM ($CD45RA^-CCR7^+$) to TEMRA ($CD45RA^+CCR7^-$) and of TEM ($CD45RA^-CCR7^-$) to TEMRA ($CD45RA^+CCR7^-$) will be assessed in BM and peripheral blood using flow cytometry. More recent preclinical data suggest that the frequency of $CD8^+$, and $CD8^+/T_{regs}$ are two important predictive markers for anti-PD-1 therapy (Twyman-Saint Victor *et al*., 2015). Additionally, this study also demonstrated that exhausted $CD8^+$ T-cells defined by the expression of PD-1 and Eomes are reinvigorated, which can be defined by increased Ki67 and GzmB, following anti-PD-1 and anti-CTLA-4 treatment in a murine model. CD47 is capable of interacting with its receptor signal-regulatory protein alpha (SIRPα) on macrophages to negatively regulate phagocytosis and blockade of CD47 expression results in phagocytosis of red blood cells (RBCs) (Oldenborg *et al*., 2000). CD47 functions as a 'don't eat me' signal to ensure that autologous cells are not inappropriately phagocytosed. Intriguingly, two recent studies revealed that CD47 is upregulated on leukemia cells and represents an adverse prognosis factor in AML (Jaiswal *et al*., 2009; Majeti *et al*., 2009). The 'eat me' signal ecto-CRT was shown to correlated inversely with the expression of the 'don't eat me' signal CD47 in AML blasts (Wemeau *et al*., 2010). We will determine whether other exploratory immune biomarkers predict the response to anti-PD-1 Ab therapy in combination with chemotherapy by assessing immune cell lineage markers as well as functional markers in fresh BM and blood from AML patients using high throughput mass spectrometry(CyTOF).

2.5.4 Multiplex Immunoflorescence (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 using Multiplex Immunoflorescence (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.

2.5.5 Dynamic change of immune cell subsets:

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). 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).

2.5.6 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 bone marrow aspirate in collaboration with Dr. Sacha Gnjatic at Mount Sinai CIMAC.

2.5.7 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.

2.5.8 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.

2.5.9 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.

2.5.10 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. 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 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 on clinical outcomes and toxicity.

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Newly diagnosed and pathologically-confirmed AML, confirmed by a bone marrow aspirate and/or biopsy and/or peripheral blood with ≥20 % myeloid blasts. 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 that is arising from prior myelodysplastic syndrome [MDS] as well as t-AML) are also allowed. Clarifications: . AML arising from myeloproliferative neoplasms (MPN), MPN/MDS overlap (including chronic myelomonocytic leukemia [CMML]) or another myeloid malignancy are NOT 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 (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. In any case, results from FISH or karyotype should show if CBF abnormalities are present by time of randomization as the presence of CBF abnormalities is a required stratification factor.

3.1.2. Age ≥18 and ≤75 years.

Because no dosing or AE data are currently available on the use of Pembrolizumab (MK-3475) in patients <18 years of age, children are excluded from this study, but will be eligible for future pediatric trials.

3.1.3. ECOG performance status ≤ 2, see Appendix A.

3.1.4. The patient has to be eligible to receive intensive "7+3" induction chemotherapy as judged by the treating physician.

3.1.5. Prior use of hypomethylating agents (HMA), lenalidomide, erythropoiesis-stimulating agents (ESAs), and growth factors is allowed if used to treat prior MDS. AML must be previously untreated except as outlined below (hydroxyurea, or ATRA, or leukapheresis). 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/leukapheresis allowed for control of hyperleukocytosis but hydroxyrea must be discontinued day prior to start of chemotherapy.

3.1.7. Patients must have adequate organ and marrow function as defined below within 3 days prior to the first day of 7+3.

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	
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
Coagulation	
International normalized ratio (INR) or prothrombin time (PT)	≤1.5 × ULN unless patient is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
Activated partial thromboplastin time (aPTT)	≤1.5 × ULN unless patient is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
[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.	

3.1.8. Patients with a known history of being 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.

3.1.9. 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.

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

3.1.11. Patients with known history or current symptoms of cardiac disease, or history of treatment with cardiotoxic agents, should have a clinical risk assessment of cardiac function using the

New York Heart Association Functional Classification. To be eligible for this trial, patients should be class 2B or better.

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 7+3 treatment. 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 12 consecutive months (*i.e.*, has had menses at any time in the preceding 12 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 with female partners 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 a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin or squamous cell carcinoma of the skin that has undergone potentially curative therapy or *in situ* cervical cancer.

Prior treatment with the following is not allowed:

- Patients who have received anthracyclines for treatment of a prior, unrelated, curatively-treated malignancy which would limit their ability to receive 7 + 3 chemotherapy treatment on study.
- Anti-PD-1, anti-PD-L1, or anti-PD-L2, for a prior, unrelated, curatively-treated malignancy, within last 3 months of enrollment in the study.
- Anti-cancer mAb within 4 weeks, for a prior, unrelated, curatively-treated malignancy, 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.
- Experimental treatment within 4 weeks prior to study registration.

3.2.2. Patients who have had chemotherapy (except hydroxyurea and all trans retinoic acid [ATRA] which are allowed but have to be stopped the day before induction therapy starts), targeted small molecule therapy (aside from imatinib, dasatinib, or nilotinib), or curative-intent radiotherapy within 4 weeks (6 weeks for nitrosoureas or mitomycin C), for a prior curatively treated malignancy, prior to entering the study.

3.2.3. Patients who have received prior anthracyclines not to exceed 150 mg/m2 of daunorubicin or equivalent for treatment of a prior, unrelated, curatively-treated malignancy which would limit their ability to receive 7 + 3 chemotherapy treatment on study.

3.2.4. Patients with a cardiac ejection fraction less than 50% as determined by Echocardiogram or MUGA scan.

3.2.5. 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).

3.2.6. Patients who have *FLT3*-mutated AML.
FLT3-ITD or TKD mutations are defined as a mutation with a ratio of mutant to wild-type allele ≥ 0.05 or variant allele fraction of ≥ 5% by PCR or next generation sequencing from either bone marrow or peripheral blood.
Note 1: FLT3, karyotype, or FISH results from bone marrow or peripheral blood that were performed up to 3 weeks before initiation of trial therapy are acceptable for eligibility determination or therapy stratification as long as they are performed in a CLIA certified laboratory. Note 2: Patients are stratified based on age (younger than 65 *vs*. 65 and older),, presence of core-binding abnormalities by FISH or karyotype (yes/no), and by having t- AML or AML arising from prior/antecedent MDS (yes/no).

3.2.7. Patients who have not recovered from AEs due to prior anti-cancer therapy (*i.e.*, have not returned to baseline or 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.

3.2.8. 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.

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

3.2.10. Current use of corticosteroids.

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) is permitted.

3.2.11. Patients who underwent prior allogenic transplant

3.2.12. 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.

3.2.13. 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.

3.2.14. Patients 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.

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

3.2.16. Patients with active, uncontrolled infection as deemed by the treating investigator. .

3.2.17. Patients with a known history of active TB (*Bacillus Tuberculosis*).

3.2.18. Patients with uncontrolled intercurrent illness.

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

3.2.20. 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 (MK-3475), breastfeeding should be discontinued if the mother is treated with Pembrolizumab (MK-3475). These potential risks may also apply to other agents used in this study.

3.2.21. Patient who have 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.

3.2.22. 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.

3.2.23. Patients with clinically significant disseminated intravascular coagulation (DIC), which cannot be managed with supportive care including transfusions, as assessed by treating physician, will be excluded from study.

3.2.24. Patients with no bone marrow involvement will be excluded (*i.e.*, those with only extramedullary disease).

3.2.25. Patients with acute promyelocytic leukemia will be excluded.

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 require sponsors to select qualified investigators. National Cancer Institute (NCI) policy requires all individuals contributing to NCI-sponsored trials to register with their qualifications and credentials 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

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 IRBManager to indicate their intent to open the study locally. The NCI CIRB's approval of the SSW is automatically communicated to the CTSU Regulatory Office, but sites are required to contact the CTSU Regulatory Office at CTSURegPref@ctsu.coccg.org to establish site preferences for applying NCI CIRB approvals across their Signatory Network. Site preferences can be set at the network or protocol level. Questions about establishing site preferences can be addressed to the CTSU Regulatory Office by emailing the email address above or calling 1-888-651-CTSU (2878).

In addition, the Site-Protocol PI (*i.e.*, the investigator on the IRB/REB approval) must meet the following 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 Federal wide 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, and protocol number 10300,

- 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 10300 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 ❼ Regulatory ❼ 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 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.

4.3.3 OPEN/IWRS Questions?

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

Theradex has developed a Slot Reservations and Cohort Management User Guide, which is

available on the Theradex website: http://www.theradex.com/clinicalTechnologies/?National-Cancer-Institute-NCI-11. This link to the Theradex website is also on the CTSU website OPEN tab. For questions about the use of IWRS for slot reservations, contact the Theradex Helpdesk at 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 SPECIALSTUDIES

5.1 Summary Table for Specimen Collection

Time Point	Specimen[2]	Send Specimens To:
Baseline [1]		
	• 5 mL bone marrow aspirate (mandatory)	Local CLIA Labs
	• 10 mL bone marrow aspirate (mandatory) • 10 mL blood in EDTA lavender top vacutainer tube (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
	• 1 bone marrow biopsy core (processed to FFPE blocks) (mandatory) • 16 mL bone marrow aspirate sent unprocessed in EDTA tubes (mandatory) • 30 mL blood in sodium heparin Green top vacutainer tubes (mandatory) • Snap Frozen Skin Punch Biopsy • Stool (OMNIgene-GUT kit)	NCI Early-Phase and Experimental Clinical Trials Biospecimen Bank (EET Biobank)
Induction therapy phase – Between Days 14 – 21		
	• 10 mL bone marrow aspirate (mandatory) • 10 mL blood in EDTA lavender top vacutainer tube (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
End of induction phase		

	• 10 mL bone marrow aspirate (mandatory) • 10 mL blood in EDTA lavender top vacutainer tube (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
Consolidation therapy phase – Post Cycle 1		
	• 10 mL bone marrow aspirate (mandatory) • 10 mL blood in EDTA lavender top vacutainer tube (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)	EET Biobank
End of consolidation phase [1]		
Time Point	**Specimen[2]**	**Send Specimens To:**
	• 10 mL bone marrow aspirate (mandatory) • 10 mL blood in EDTA lavender top vacutainer tube (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
	• 1 bone marrow biopsy core (processed to FFPE blocks) (mandatory) • 16 mL bone marrow aspirate sent unprocessed in EDTA tubes (mandatory) • 30 mL blood in sodium heparin Green top vacutainer tubes (mandatory)	EET Biobank
Maintenance therapy/observation phase – Every 3 months (±7 days)		
	• 10 mL bone marrow aspirate (optional – only if patients have a standard-of-care biopsy that coincides with this collection time point.) • 10 mL blood in EDTA lavender top vacutainer tube (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
After 6 months of Treatment		
	• 1 bone marrow biopsy core (processed to FFPE blocks) (optional) • 16 mL bone marrow aspirate sent unprocessed in EDTA tubes (optional) • 30 mL blood in sodium heparin Green top vacutainer tubes (mandatory)	EET Biobank
After 1 year of Treatment		
	• 1 bone marrow biopsy core (processed to FFPE blocks) (optional) • 16 mL bone marrow aspirate sent unprocessed in EDTA tubes (optional) • 30 mL blood in sodium heparin Green top vacutainer tubes (mandatory)	EET Biobank
Relapse		
	• 1 bone marrow biopsy core (processed to FFPE blocks) (optional) • 16 mL bone marrow aspirate sent unprocessed in EDTA tubes (mandatory) • 30 mL blood in sodium heparin Green top vacutainer tubes (mandatory)	EET Biobank
End of maintenance/observation phase*		
	• 10 mL bone marrow aspirate (mandatory) • 10 mL blood in EDTA lavender top vacutainer tube (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

[1] Sample priority in cases of limited aspirate will be for MRD testing by flow cytometry and DS (one tube of 10 cc or whatever amount can be extracted). Every effort should be made to get an aspirate for MRD assessment at all the above required time points, but in cases were 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 as described above.

[2] 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.

[3] For skin punch biopsies, the **Skin Punch Biopsy Verification Form (Appendix F)** must be completed and sent with the tissue to the EET Biobank.

*For patients who do not progress.

5.2 Specimen Procurement Kits and Scheduling

5.2.1 Specimen Shipping Kits

Kits for the submission of frozen skin punch tissue, and for 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, and jradich@fredhutch.org to request shipment of Streck tubes.
- 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.
- Specimens submitted frozen such as skin punch biopsy tissue can be collected on any day but must be stored frozen until shipped to the EET Biobank on Monday through Thursday. In the event that frozen specimens cannot be shipped immediately, they must be maintained in a -70°C to -80°C freezer.
- Stool samples may be collected any day and may be shipped on Monday through Thursday.

5.2.3. Scheduling of Specimen Collections for Brent Wood's lab, 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.
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 when available. 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 the 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 specimens (including whole blood or bone marrow, 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.
- 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.*, formalin jar):

- 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
- Collection date

5.3.2.3 Stool Specimen Labels

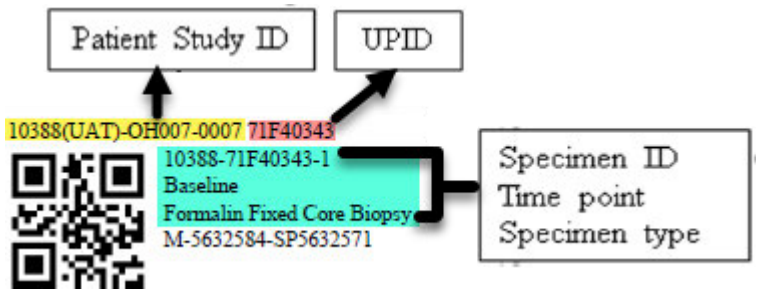
Include the following on all stool specimens or containers (*e.g.*, vials):

- 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 and optional time. The last line on the example label is for the handwritten date and optional time.

5.3.3. Overview of Process at Treating Site

5.3.3.1 OPEN Registration

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

Registration with eligibility specimen analysis:

1. Site enters first step data into OPEN.
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 first step registration data, including the IDs and a TAC of "NOT REG" directly to Rave.
4. The specimen tracking system in Rave is utilized for the specimen that contributes to eligibility determination.
5. Site enters second and any subsequent step data into OPEN including results of specimen analysis.
6. IWRS receives all data from OPEN, then sends it onto Rave with either the treatment TAC or a TAC of "SCRN FAIL".
7. In addition to the specimen tracking forms completed to determine eligibility, data entry for screen failure patients should include Histology and Disease, all forms in the Baseline folder, any lab forms connected to eligibility determination, and Off Treatment/Off Study.

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, 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.
- Apply an extra specimen label to *each* report before scanning. Return to the **Histology and Disease** form to upload any initial Pathology, Radiology, Bone Marrow, Molecular Reports (up to 4), Surgical (or Operative) reports and Tissue Biopsy Verification form (when applicable). 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 Skin Punch Biopsy Verification Form (when applicable). Uploaded reports should have protected health information (PHI) data, like name, date of birth, mailing address, medical record number or social security number (SSN), redacted. Do not redact SPID, block number, diagnosis, or relevant dates (such as collection date) and include the UPID and patient study ID on each document (either by adding a label or hand-writing).

Step 3: Complete specimen data entry.

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

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

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

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

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

Step 6: Send email notification.

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

Step 7: Ship the specimen(s).

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

5.4 Specimen Collection and Processing

5.4.1. Pre-Analytic 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 captures the chronological order in which the biopsy cores were obtained.

5.4.2 Bone Marrow Aspirate Collection

5.4.2.1 Collection of Bone Marrow Aspirate

Bone Marrow Aspiration Procedure:

1. Label EDTA tubes according to the instructions in section 5.3.2.

2. For time points with submissions to both Brent Wood's lab, Children's Hospital Los Angeles, and the EET Biobank: Obtain 26 mL of bone marrow aspirate into a single tube and aliquot 10 mL in the first EDTA tube (mandatory, to ship to Dr. Wood's Lab) and aliquot 16 mL into two EDTA tubes (~8 mL in each, to ship to EET Biobank) (mandatory). Mix the EDTA tubes, by inversion, 8-10 times. Ship the first 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. The second and third tubes will be sent to the EET Biobank for immune correlative studies.**

3. For time points with only shipping to Dr. Wood's Lab: obtain 10 mL of bone marrow aspirate and aliquot into an EDTA tube (optional during the maintenance phase). 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.**

4. For time points submissions sent only to the EET Biobank: obtain 16 mL of bone marrow aspirate and aliquot ~8 mL into each EDTA tube (optional). Mix the EDTA tubes, by inversion, 8-10 times. **This tube will be sent to the EET Biobank for immune correlative studies.**

5. Ship on the day of collection. If liquid specimens cannot be shipped on the day of collection, then store tubes at 4ºC until shipment.

**For baseline bone marrow, obtain an additional 5 mL of bone marrow aspirate and aliquot in EDTA tube (to be sent to local CLIA labs 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 Bone Marrow Core Biopsy Collection and Processing

5.4.3.1 Collection of Bone Marrow Core Biopsy

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

5.4.3.2 Processing of Bone Marrow Core Biopsy

Each bone marrow core biopsies should be processed into separate FFPE blocks (1 core per block) 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 E).
4. Sites should follow embedding protocols where the total processing time from 70% ethanol to block embedding ***exceeds 4 hours.***

5.4.4 Flash Freezing of Skin Punch Biopsies

1. Tissue should be frozen as soon as possible. Optimally, freeze within 30 minutes from biopsy collection.
2. Prior to tissue collection:
    a. Label cryovial(s) according to instructions in Section 5.3.2.2.
    b. Place cryovial(s) on dry ice to freeze. The vials should appear frosty when ready.
3. Obtain one ≥5mm skin punch biopsy.
4. Immediately place tissue in foil and allow to completely freeze (using either direct contact with dry ice, or liquid nitrogen vapor).
5. Gently remove the frozen tissue from the foil. If the tissue is sticking to the foil, then gently run a finger over the back of the foil to loosen the tissue.
6. Using clean forceps place each tissue core in a separate pre-chilled cryovial. Tissue should move freely in the vial.
7. Place the tissue in a -70 to -80°C freezer. Keep frozen until shipment to the EET Biobank.

5.4.5 Blood Collection`

5.4.5.1 Collection of Blood in EDTA Tubes for Shipment to Brent Wood's lab, Children's Hospital Los Angeles

1. Label EDTA tubes according to the instructions in section 5.3.1.
2. Collect 10 mL of blood in 1 EDTA tubes 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, Children's Hospital Los Angeles** 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.

5.4.5.2 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 5.5.

5.4.5.3. Collection of Blood in Green Top Sodium Heparin Tubes

1. Label Green Top tubes according to the instructions in section 5.3.1
2. Collect 30 mL of blood in 3 Heparin tubes and gently invert tube 8-10 times to mix.
3. Identify the peripheral blood tube by writing the letters "PB" on the tube.
4. Blood samples **being sent to the EET Biobank** 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.
7. An external sample label should be fixed to the shipping container to alert the Biorepository of ***blood*** sample collection ***time and date*** (this helps to identify and prioritize received samples that have processing time requirements).
8. Blood should be shipped ambient FedEx Priority Overnight to the biorepository where it is processed the day of receipt ***within 24 hours of collection (not to exceed 48 hours).***

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

Baseline and "Consolidation therapy phase – Post Cycle 1" timepoints will use OMNIgene GUT kits (OMR-200.100—shipped ambient) which include a DNA stabilizing solution. Collection kits will include directions for specimen collection and shipment as well as collection and shipping supplies.

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 prior to being provided to the patient.
2. The patient will collect the stool sample and return the sample to the 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.
3. The stool and a shipping manifest should be shipped on day of collection (whenever possible) according to instructions provided in the kit and section 5.5.1.2.
4. Collection site staff should not ship any samples that do not utilize the collection kit properly.

5.5 Shipping Specimens from Clinical Site to the EET Biobank

5.5.1 General Shipping Information

When kits are provided, the shipping container sent with kit contents should be used to ship specimens to the EET Biobank. In winter months, please include extra insulation, such as bubble wrap, inside the shipping container.

5.5.1.1 Required Forms for Specimen Submissions:

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

Specimen	**Required Forms**
Bone Marrow Aspirate or Biopsy	1.Shipping List 2.Corresponding Bone Marrow Report
Other (blood, blood product, stool)	1. Shipping List
Skin Punch Biopsy	1.Shipping List 2.Skin Punch Biopsy Verification Form (Appendix F)

5.5.2. Specimen Shipping Instructions

FFPE tissue and frozen specimens may be shipped on Monday through Thursday.

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

Frozen specimens such as frozen skin punch tissue should be shipped on dry ice in an insulated shipping container provided by the EET Biobank. Keep frozen in a -70ºC to -80ºC freezer until

shipment to the EET Biobank.

5.5.2.1. Shipping Ambient Tissue, Blood and Bone Marrow

1. Before packaging specimens, verify that each specimen is labeled according to the instructions above and that the lids of all primary receptacles containing liquid are tightly sealed
2. Place the specimens in zip-lock bags. Use a separate bag for each specimen type.
3. Place specimens 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 and corresponding reports such as the bone marrow report into the shipping container. In winter months, please include extra insulation, such as bubble wrap, inside the shipping container, to prevent specimens from freezing.
6. Place the lid 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.

5.5.2.2 Shipping Frozen Specimens in a Single-Chamber Kit

1. Before packaging specimens, verify that each specimen is labeled according to the instructions in 5.3 and that lids of all primary receptacles are tightly sealed.
2. Place the specimens in zip-lock bags. Use a separate zip-lock bag for each specimen type and time point.
3. Place the zip-lock bags in the biohazard envelope containing absorbent material. Expel as much air as possible and seal securely.
4. Put the secondary envelope into a Tyvek envelope. Expel as much air as possible and seal securely.
5. Place frozen specimens in the kit compartment with dry ice. Layer the bottom of the compartment with dry ice until it is approximately one-third full. Place the frozen specimens on top of the dry ice. Cover the specimens with additional dry ice until the compartment is almost completely full. When packaging specimens, ensure that you leave enough room to include at least 5 pounds of dry ice in the shipment.
6. Insert a copy of the required forms into a plastic bag and place in the kit chamber.
7. Place the Styrofoam lid on top to secure specimens during shipment. Do not tape the inner chamber shut.
8. Close the outer lid of the Specimen Procurement Kit and tape it shut with durable sealing tape. Do not completely seal the container.
9. Complete a FedEx air bill and attach to top of shipping container.
10. Complete a dry ice label.
11. Attach the dry ice label and an Exempt Human Specimen sticker to the side of the

shipping container.

12. Ship specimens via overnight courier to the address below. FedEx Priority Overnight is strongly recommended to prevent delays in package receipt.

5.5.2.3. Shipping Stool in an Ambient Shipper

The clinical site will ship each specimen to the EET 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 specimen are provided in the kit.

5.5.3. 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.

5.5.4. 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, 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.
1. Specimen must be placed in a leak proof vacutainer.
2. Multiple fragile vacutainers must be individually wrapped or separated to prevent contact.

3. 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.
4. The Ziploc bag must be labeled with the universal biohazard symbol.
5. 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.
6. The Ziploc bag must be placed into a rigid outer package with suitable cushioning materials.
7. The outer packaging must be clearly and durably marked with the words "Diagnostic Specimen" and 2-inch diamond with "UN3373" inside of the diamond.
8. The outer packaging must be marked with the name, address, and phone number of both sender and recipient.
9. Specimen should be shipped with a cold pack (do not allow to freeze).

The Department of Pathology and Laboratory Medicine is open Monday-Friday 8:00-4:30. Do not draw specimens on Fridays for delivery on Saturday. The laboratory will not be open to accept and test specimens.

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 2-290
Los Angeles, CA 90027
Phone: (877) 543-9522

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

5.6.1.3. Contact Information for Assistance

Contact the Department of Pathology and Laboratory Medicine (877) 543-9522 or plmclinicalimmunologylaboratory@chla.usc.edu for assistance.

5.7 Shipping of Specimens from Clinical Site to Radich laboratory, Fred Hutchinson Cancer Research Center

5.7.1.1 Specimen Shipping Instructions

1 Specimen should be received by the Radich Laboratory, Fred Hutchinson Cancer Research Center within 5 days of collection, ideally no later than 48 hours after 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 Radich Laboratory, Fred Hutchinson Cancer Research Center is open Monday-Friday. Do not draw specimens on Fridays for delivery on Saturday. The laboratory will not be open to accept and test specimens. Exception can be made on a case-by-case basis for Friday shipping but should be discussed in advance of performing screening samples with the study chair and approved.

5.7.1.2 Shipping Address

Send FedEx tracking number to:

lbeppu@fredhutch.org and

jradich@fredhutch.org

Send the specimens FedEx Priority Overnight to:

Radich Laboratory
Fred Hutchinson Cancer Research Center
1100 Fairview Avenue North
Room D4-385
Seattle, WA 98109

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

5.7.1.3 Contact Information for Assistance

Contact the Radich Laboratory at 206-667-6966 or lbeppu@fredhutch.org.

5.8 Biomarker Plan

List of Biomarker Assays in Order of Priority

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

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	MultiplexIF CLIA: N	Exploratory Correlate with clinical response	FFPE Bone Marrow Core Biopsy, decalcified	Baseline, end of consolidation, after 6 months of treatment, after 1 year of treatment, and at relapse	M/O (optional for the after 6 months of treatment and after1 yearof treatment time points)	CIMAC Ignacio Wistuba iiwistuba@mdanderson.org Dr. Gheath Al-Atrash galatras@mdanderson.org Dr. Cara Haymaker CHaymaker@mdanderson.org
Bone Marrow Aspiration Biomarkers							
1	CBF gene alterations	Cytogenetics or Fluorescence In situ Hybridization (FISH) testing CLIA: Y	Integral Stratification factor	Bone marrow aspirate	Baseline and as clinically indicated	M	Local CLIA labs
2	FLT3 Mutations	Molecular FLT3 assay (either *FLT3*-PCR testing or as a part of Next generation sequencing panel) CLIA: Y	Integral Exclusion factor	N/A (collected as part of the CBF gene alterations assay)	Baseline and as clinically indicated	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 (MFC) CLIA: Y	Integral Primary Endpoint	Bone marrow aspirate	Baseline Consolidation therapy phase: Post Cycle 1.	M	Department of Pathology and Laboratory Medicine Children's Hospital Los Angeles Brent Wood bwood@chla.usc.edu
4	MRD Status	Duplex Sequencing (DS) CLIA: N	Exploratory Exploratory Endpoint	Bone marrow aspirate	Baseline Induction therapy phase: Days 14 and end of induction. Consolidation therapy phase: Post Cycle 1 and end of consolidation. Maintenance therapy phase: Every 3 months (BM is optional but if done a BM sample should be sent) End of maintenance/observation phase for patients who do not progress.	M/O (optional during the maintenance phase)	To be shipped from Brent Wood's lab after completion of flow cytometry studies Hematopathology Laboratory at Fred Hutchinson Cancer Research Center Jerald Radich jradich@fhcrc.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	Immune cell subset Analysis (CyTOF)	CyTOF CLIA: N	Exploratory Correlate with clinical responses	Mononuclear cells from Bone marrow aspirate in EDTA	Baseline, end of consolidation, after 6 months of treatment, after 1 year of treatment, and at relapse	M/O (optional for the after 6 months of treatment and after1 yearof treatment time points)	MDACC CIMAC Dr. Ignacio I Wistuba iiwistuba@mdanderson.org Dr. Gheath Al-Atrash galatras@mdanderson.org Dr. Cara Haymaker CHaymaker@mdanderson.org
6	Olink	Olink CLIA: N	Exploratory Correlate with clinical responses	Plasma from Bone marrow aspirate in EDTA	Baseline, end of consolidation, after 6 months of treatment, after 1 year of treatment, and at relapse	M/O (optional for the after 6 months of treatment and after 1 year of treatment time points)	Mt. Sinai CIMAC Sasha Gnjatic Sacha.gnjatic@mssm.edu
7	Whole Exome Sequencing	WES (tumor) CLIA: N	Exploratory Correlate with clinical response	DNA from Mononuclear cells from bone marrow aspirate	Baseline and at relapse	M	MDACC CIMAC Dr. Ignacio I Wistuba iiwistuba@mdanderson.org Dr. Gheath Al-Atrash galatras@mdanderson.org Dr. Cara Haymaker CHaymaker@mdanderson.org
	Whole Exome Sequencing	WES (Germline) CLIA:N	Exploratory Correlate with clinical response	DNA from Skin punch biopsies for germline control	Baseline	M	MDACC CIMAC Dr. Ignacio I Wistuba iiwistuba@mdanderson.org Dr. Gheath Al-Atrash galatras@mdanderson.org Dr. Cara Haymaker CHaymaker@mdanderson.org

8	RNA-Seq	RNA-Seq CLIA: N	Exploratory Correlate with clinical response	RNA from mononuclear cells from bone marrow aspirate in EDTA	Baseline and at relapse	M	MDACC CIMAC Dr. Ignacio I Wistuba iiwistuba@mdanderson.org Dr. Gheath Al-Atrash galatras@mdanderson.org Dr. Cara Haymaker CHaymaker@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
9	TCR-Seq	TCR-Seq CLIA: N	Exploratory Correlate with clinical response	DNA from mononuclear cells from bone marrow aspirate in EDTA	Baseline, end of consolidation, after 6 months of treatment, after 1 year of treatment, and at relapse	M/O (optional for the after 6 months of treatment and after1 yearof treatment time points)	Adaptive Biotechnologies working with MDACC CIMAC Dr. Ignacio I Wistuba iiwistuba@mdanderson.org Dr. Gheath Al-Atrash galatras@mdanderson.org Dr. Cara Haymaker CHaymaker@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 (DS) CLIA: N	Exploratory Exploratory Endpoint	Peripheral blood (EDTA)	Baseline Induction therapy phase: Days 14 and end of induction. Consolidation therapy phase: Post Cycle 1 and end of consolidation. Maintenance therapy phase: Every 3 months End of maintenance/observation phase for patients who do not progress.	M	To be shipped from Brent Wood's lab after completion of flow cytometry studies Molecular Oncology Laboratory at Fred Hutchinson CancerResearch Center Jerald Radich jradich@fhcrc.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							
2	cfDNA	Duplex Sequencing (DS) CLIA: N	Exploratory Exploratory Endpoint	Peripheral blood (Streck)	Baseline Induction therapy phase: Days 14 and end of induction. Consolidation therapy phase: Post Cycle 1 and end of consolidation. Maintenance therapy phase: Every 3 months	M	To be shipped to Radich laboratory, Fred Hutchinson Cancer Research Center Molecular Oncology Laboratory at Fred Hutchinson CancerResearch Center Jerald Radich jradich@fhcrc.org
3	Immune cell subset analysis	CyTOF CLIA: N	Exploratory Correlate with clinical responses	PMBCs from blood collected in heparin	Baseline, end of consolidation, after 6 months of treatment, after 1 year of treatment, and at relapse	M	MDACC CIMAC Dr. Ignacio I Wistuba iiwistuba@mdanderson.org Dr. Gheath Al-Atrash galatras@mdanderson.org Dr. Cara Haymaker CHaymaker@mdanderson.org
4	Olink	Olink CLIA: N	Exploratory Correlate with clinical responses	Plasma from blood collected in heparin (same collection as for immune cell subset analysis)	Baseline, end of consolidation, after 6 months of treatment, after 1 year of treatment, and at relapse	M	Mt. Sinai CIMAC Sasha Gnjatic Sacha.gnjatic@mssm.edu
5	TCR-seq	TCR-Seq CLIA: N	Exploratory Correlate with clinical responses	DNA from PBMCs from blood collected in EDTA or sodium heparin tubes	Baseline, end of consolidation, after 6 months of treatment, after 1 year of treatment, and at relapse	M	Adaptive Biotechnologies working with MDACC CIMAC Dr. Ignacio I Wistuba iiwistuba@mdanderson.org Dr. Gheath Al-Atrash galatras@mdanderson.org Dr. Cara Haymaker

							CHaymaker@mdanderson.org
Stool based biomarkers							
1	Gut microbiome ch characterizati on	16sRNA V4 region sequencing of bacterial genomic DNA	• Exploratory Correlate with clinical response	Stool (OMNIgene-GUT)	• Baseline • Post Cycle 1 Consolidation	M	MDACC CIMAC Gheath Al-Atrash

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

5.9 Integral Laboratory Studies

5.9.1 CBF gene alterations

5.9.1.1 Specimen Receipt and Processing at Local Labs

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

5.9.1.2 Site Performing Correlative Study

This study will be conducted by local labs.

5.9.2 *FLT3* Mutations

5.9.2.1 Specimen Receipt and Processing at Local Labs

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

5.9.2.2 Site Performing Correlative Study

This study will be conducted by local labs.

5.9.3 MRD Status by Multicolor Flow Cytometry

5.9.3.1 Specimen Receipt and Processing at the Department of Pathology and Laboratory Medicine Children's Hospital Los Angeles

Bone marrow aspirate 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. Brent Wood at the Children's Hospital Los Angeles

6.0 Exploratory/Ancillary Correlative Studies

6.1 MRD Status by Duplex Sequencing

6.1.1 Specimen Receipt and Processing at the Department of Pathology and Laboratory Medicine Children's Hospital Los Angeles

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

6.1.2.1 Site(s) Performing Correlative Study

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

6.1.3 Multiplex Immunofluorescence (MIF)

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

The EET Biobank will receive and barcode FFPE core bone marrow biopsy blocks, and will store them at room temperature until distribution.

When a request is received from CIMAC/NCI, the EET Biobank will cut slides from the blocks and ship the slides to MDACC CIMAC.

6.1.3.2 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), Dr. Gheath Al-Atrash (Email: galatras@mdanderson.org), and Dr. Cara Haymaker (Email: CHaymaker@mdanderson.org) (MDACC CIMAC Co-PI's).

Mei Jiang
Translational Molecular Pathology- TMP-IL-Pathology Lab
UT MD Anderson Cancer Center
Life Science Plaza Building
2130 W. Holcombe Blvd
Houston, TX 77030
location: LSP9.3003 (shipping)

6.1.4 Immune Cell Subset Analysis CYTOF

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

The EET Biobank 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 stored in liquid nitrogen vapor phase.

6.1.4.2 Site(s) Performing Correlative Study

This study will be performed at the MDACC CIMAC under Dr. Ignacio I Wistuba (iiwistuba@mdanderson.org), Dr. Gheath Al-Atrash (Email: galatras@mdanderson.org), and Dr. Cara Haymaker (Email: CHaymaker@mdanderson.org) (MDACC CIMAC Co-PI's).

UT MD Anderson Cancer Center
Room 2SCR2.3219
7435 Fannin St
Houston, TX 77054
c/o Karen Millerchip

6.1.5 O-link Cytokine panel

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

The EET Biobank 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 stored in liquid nitrogen vapor phase. The same blood samples collected in heparin tubes from the immune cell subset analysis will also be used for this assay.

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

6.1.5.2 Site(s) Performing Correlative Study

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

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

6.1.6 Whole Exome Sequencing

6.1.6.1 Specimen(s) Receipt and Processing at the EET Biobank for Bone marrow aspirate

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.

DNA and RNA will be co-extracted from bone marrow mononuclear cells. An aliquot of DNA will be used for this assay. The same blood samples collected in heparin tubes from the immune cell subset analysis can also be used for this assay if a bone marrow aspirate sample cannot be obtained.

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

The EET Biobank will extract germline DNA from the skin punch biopsies upon receipt. The stock DNA tube will be stored in a -80°C freezer. The EET Biobank will ship a DNA aliquot to MDACC CIMAC.

6.1.6.3 Site(s) Performing Correlative Study

This study will be performed at the MDACC CIMAC under Dr. Ignacio I Wistuba (iiwistuba@mdanderson.org), Dr. Gheath Al-Atrash (Email: galatras@mdanderson.org), and Dr. Cara Haymaker (Email: CHaymaker@mdanderson.org) (MDACC CIMAC Co-PI's).

UT MD Anderson Cancer Center
Room 4SCR5.2085
1881 East Rd.
Houston, TX 77054
c/o Latasha Little,
Srilekha Manali Rameshkumar

6.1.7 RNA-Seq

6.1.7.1 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.

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

6.1.7.2 Site(s) Performing Correlative Study

This study will be performed at the MDACC CIMAC under Dr. Ignacio I Wistuba (iiwistuba@mdanderson.org), Dr. Gheath Al-Atrash (Email: galatras@mdanderson.org), and Dr. Cara Haymaker (Email: CHaymaker@mdanderson.org) (MDACC CIMAC Co-PI's).

UT MD Anderson Cancer Center
2130 W Holcombe Blvd.
Life Science Plaza Suite 910, LSP9.3003
Houston, TX77030-3306

c/o Dzifa Duose & Jessica Lan

6.1.8 TCR-Seq

6.1.8.1 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 this assay.

Nucleic acids 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).

6.1.8.2 Site(s) Performing Correlative Study

This study will be performed by Adapitve Biotechnologies working with the MDACC CIMAC under Dr. Ignacio I Wistuba (iiwistuba@mdanderson.org), Dr. Gheath Al-Atrash (Email: galatras@mdanderson.org), and Dr. Cara Haymaker (Email: CHaymaker@mdanderson.org) (MDACC CIMAC Co-PI's).
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Attn: BSM Department
Adaptive Biotechnologies
1165 Eastlake Ave East
Seattle, WA 98109

6.1.9 Gut microbiome

6.1.9.1 Specimen(s) receipt and processing at the EET Biobank

Upon receipt of the sample in the OMNIgene-GUT kits, the stool sample will be processed, aliquoted, and stored in a -80°C freezer.

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

6.1.9.2 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).

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

7. TREATMENT PLAN

7.1 Agent Administration

Treatment will be administered on an inpatient and outpatient basis. Reported AEs 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.

7.1.1 Induction therapy phase

Eligible patients will be randomized before proceeding to 7+3, or within one week of starting 7+3 in 1:1 ratio, to 7+3 alone or 7+3+ Pembrolizumab (MK-3475). As shipment of pembrolizumab to site can only be ordered after randomization, every effort should be done to randomize the patient as soon as possible. See Section 9.1 for agent ordering information. If patient is randomized after initiation of 7+3 therapy, the patient should continue to meet all eligibility criteria up to randomization date. Planned stratification factors include 1) age (younger than 65 *vs*. 65 and older), 2) cytogenetics **by FISH or metaphase karyotype** (presence *vs.* absence of core binding factor inversions and translocations), and 3) **t- AML or AML arising from prior/antecedent MDS (yes/no**). All patients will receive cytarabine at 100 mg/m^2/day continuous infusion on Days 1-7 and either idarubicin 12 mg/m^2/day IV on Days 1-3 or daunorubicin 60 mg/m^2/day IV on Days 1-3. A bone marrow biopsy on Day 14 (between Days 14 and 21) will be performed to assess for chemoablation. If patients have evidence of residual leukemia (defined as per institutional practice) in their bone marrow and following assessment by repeat transthoracic echocardiography (TTE) for assessment of ejection fraction, they will receive re-induction 5+2 chemotherapy, which means patients will receive cytarabine at 100 mg/m^2/day continuous infusion on Days 1-5 and either idarubicin 12 mg/m^2/day IV or daunorubicin 60 mg/m^2/day IV on Days 1-2. Anthracycline dosing adjustments may need to be made, per institutional guidelines. Regardless of chemoablation status, on Day 8 of the induction chemotherapy and Q3W (±3 days) thereafter, half of the patients will receive Pembrolizumab (MK-3475) IV (intervention arm). The other half will not receive Pembrolizumab (MK-3475) (control arm). At time of count recovery, defined as platelet count more than 50,000 or neutrophil count more than 500, patients will undergo a bone marrow aspirate/biopsy in order to assess for response and MRD. If there is no count recovery, a bone marrow will have to be performed by day 42. If the bone marrow shows hypoplasia and patient remains without count recovery, a bone marrow will be performed q 2 weeks (+/- 3 days) till there is count recovery or evidence of persistent AML. If patients have a CR or a CRi, patients will continue with consolidation therapy. If patients do not achieve a CR or CRi, they will be taken off study. If Pembrolizumab is discontinued for any reason (after at least one dose of Pembrolizumab, the patient should continue on 7+3 or Re-Induction on study.

Induction Therapy Regimen Description					
Agent	**Premedications ; Precautions**	**Dose***	**Route**	**Schedule**	**Cycle# Length**
Cytarabine	Premedications/ precautions as per standard local procedures. No specific premedications required. premedication with antipyretic and antihistamine may be considered.	100 mg/m^2 Reconstitution and diluents as per standard local procedures/ per package insert. Refer to Section 8.2.1 for additional information.	Continuous IV	Days 1-7	28-35 days (4-5 weeks)
Either Daunorubicin or Idarubicin ``	Premedications/ precautions as per local standard procedures. No specific premedications required. premedication with antipyretic and antihistamine may be considered.	Either 60 mg/m^2 Daunorubicin or 12 mg/m^2 Idarubicin on Days 1, 2, and 3 Reconstitution and diluents as per standard local procedures/ per package insert. Refer to	slow IV push over 10 to 15 minutes into free-flowing side-port tubing of a rapidly infusing IV solution of D5W or NS and infuse over 15 to 30 minutes, or per institutional standard for vesicant medications.	Days 1-3	28-35 days (4-5 weeks)

		Sections 8.2.2 and 8.2.3 for additional information.			

Induction Therapy Regimen Description					
Agent	**Premedications ; Precautions**	**Dose***	**Route**	**Schedule**	**Cycle[#] Length**
Pembrolizumab (MK-3475) (For Intervention Arm only)	Premedication with antipyretic and antihistamine may be considered. No premedication with steroids	200 mg See Section 8.1 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) starting on Day 8	28-35 days (4-5 weeks)
*Dose rounding per institutional guidelines is allowed. [#]If evidence of residual leukemia in bone marrow on Day 14 bone marrow, re-induction 5+2 chemotherapy (cytarabine at 100 mg/m^2/day continuous IV on Days 1-5 and either idarubicin 12 mg/m^2/day IV or daunorubicin 60 mg/m^2/day IV on Days 1-2). Anthracycline dosing adjustments may need to be made per institutional guidelines					

7.1.2 Consolidation therapy phase

Consolidation therapy will consist of up to four cycles of infusional HiDAC and Pembrolizumab (MK-3475) (intervention arm) *vs*. HiDAC alone (control arm). Patients <60 years of age will receive 3 g/m^2 every 10-12 hours (Q12H) on Days 1, 3, and 5 (total 6 doses). Patients ≥60 years of age will receive 1.5 g/m^2 Q12H on Days 1, 3, and 5 (total 6 doses). Cycle 1 of consolidation therapy must start within 4 weeks of documenting remission status. Each cycle lasts ~28 days (up to 42 days). Patients in the intervention arm will continue to receive MK-3475 (pembrolizumab) Q3W (±3 days). Do not administer pembrolizumab concurrently with HIDAC chemotherapy Days 1-5 of each consolidation cycle. For consideration of timing of pembrolizumab, allow at least 3 hours after completion of chemotherapy prior to pembrolizumab

administration. Administration window for pembrolizumab may be extended to allow for this timing as necessary. After Cycle 1 of HiDAC, bone marrow assessment will be performed at the time of count recovery (same rules apply as post induction bone marrow in case of lack of count recovery) for formal assessment of response and MRD. Patients, who remain in CR or CRi, will be allowed to proceed to up to 3 more cycles of HiDAC and Pembrolizumab (MK-3475) (intervention arm) *vs*. HiDAC alone (control arm). Patients, who are not in CR or CRi will be taken off study. . If Pembrolizumab (MK-3475) is discontinued for any reason (after at least one dose of Pembrolizumab), the patient should continue Consolidation therapy on study.

Consolidation Therapy Regimen Description					
Agent	**Premedications ; Precautions**	**Dose***	**Route**	**Schedule**	**Cycle Length**
Pembrolizumab (MK-3475) (For Intervention Arm only)	Premedication with antipyretic and antihistamine may be considered. No premedication with steroids	200 mg See Section 8.1 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) continuous from Day 8 of Induction. For consideration of timing of pembrolizumab, allow at least 3 hours after completion of chemotherapy prior to pembrolizumab administration	28 days (±7 days)

Cytarabine[#]	Premedications/ precautions as per standard local procedures. No specific premedications required. premedication with antipyretic and antihistamine may be considered	3 g/m^2. For patients ≥60 years, dose lowered to 1.5 g/m^2 Q12H on Days 1, 3, and 5. Reconstitution and diluents as per standard local	IV over 1-3 hours every 10-12 hours	Days 1, 3, and 5	

Consolidation Therapy Regimen Description					
Agent	**Premedications ; Precautions**	**Dose***	**Route**	**Schedule**	**Cycle Length**
		procedures / per package insert. Refer to Section 8.2.1 for additional information.			
*Dose rounding per institutional guidelines is allowed.					

7.1.3 Maintenance therapy phase

Following count recovery from the last cycle of consolidation therapy, a bone marrow biopsy will be performed to assess for response and MRD. Patients, who remain in CR/CRi will continue on maintenance therapy while patients who do not remain in CR/CRi will be taken off study. Patients in CR/CRi will proceed with single-agent Pembrolizumab (MK-3475) (intervention arm) *vs*. no maintenance therapy (control arm) Q3W dosing schedule for up to 2 years, calculated from first day of maintenance therapy, according to their original randomization (*i.e*., there will be no second randomization). For patients randomized to Arm B, Pembrolizumab (MK-3475) will be administered on Day 1 (+/- 3 days) of each maintenance cycle (each maintenance cycle is 21 days). Bone marrow samples will be obtained every 3 months while

patients are receiving maintenance or in observation to assess for MRD. Patients, who are deemed to require HSCT, can forgo any remaining protocol-defined consolidation or 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 and chronic GVHD.

Maintenance Therapy Regimen Description					
Agent	**Premedications ; Precautions**	**Dose**	**Route**	**Schedule**	**Cycle Length**
pembrolizumab (MK-3475) (For Intervention Arm only)	Premedication with antipyretic and antihistamine may be considered. No premedication with steroids	200 mg See Section 8.1 for instructions on the preparation of the infusion solution.	IV over 30 minutes (between 25-40 minutes) using an <u>infusion set containing a low-protein binding 0.2 to 5 µm in-line filter.</u> See Section 8.1 for compatible infusion set materials including in-line filter.	Day 1(+/- 3 days) of each 21-day maintenance cycle	21 Days

7.1.4 CTEP IND Agent

Trial treatment of Pembrolizumab (MK-3475) will be administered Q3W starting on Day 8 of

the first induction cycle 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).

7.2 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.

7.2.1 Pembrolizumab (MK-3475) 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 thepatient.

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.
- Investigational anti-leukemia 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 study chair and 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.

7.2.2 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.

7.2.3 Recognition and Treatment of Early Immune Related Events

Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of adverse events with potential immunologic etiology are outlined below. Where appropriate, these guidelines include the use of oral or intravenous 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 MK3475 (pembrolizumab). Below represent guidelines and not required actions, but investigators are strongly encouraged to follow the below interventions.

A baseline CT scan of the chest should be considered in all patients.

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

Pneumonitis:

- For Grade 2 events, treat with systemic corticosteroids. When symptoms of Grade 2 events improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
- For Grade 3-4 events, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed. Pembrolizumab should be permanently discontinued for grade 3 or 4 pneumonitis and persistent grade 2 pneumonitis in the event of at least a possibly pembrolizumab-related pneumonitis.
- Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.

Diarrhea/Colitis:

Subjects should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood, or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus).

- All subjects who experience 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. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.
- For Grade 2 diarrhea/colitis that persists greater than 3 days, administer oral corticosteroids.
- For Grade 3 or 4 diarrhea/colitis that persists > 1 week, treat with intravenous steroids followed by high dose oral steroids.
- When symptoms of Grade 2 events improve to Grade 1 or less, steroid taper should be

started and continued over no less than 4 weeks (Grade 3-4 events may require permanent discontinuation).

Type 1 diabetes mellitus (if new onset, including diabetic ketoacidosis [DKA]) or ≥ Grade 3 Hyperglycemia, if associated with ketosis (ketonuria) or metabolic acidosis (DKA)

- For T1DM or Grade 3-4 Hyperglycemia
  - Insulin replacement therapy is recommended for Type I diabetes mellitus and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria.
  - Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.

Hypophysitis:

- For Grade 2 events, treat with corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
- For Grade 3-4 events, treat with an initial dose of IV corticosteroids followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

Hyperthyroidism or Hypothyroidism:

Thyroid disorders can occur at any time during treatment. Monitor patients for changes in thyroid function (at the start of treatment, periodically during treatment, and as indicated based on clinical evaluation) and for clinical signs and symptoms of thyroid disorders.

Grade 2 hyperthyroidism events (and Grade 2-4 hypothyroidism):

In hyperthyroidism, non-selective beta-blockers (*e.g*. propranolol) are suggested as initial therapy.

In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyroinine, is indicated per standard of care.

- Grade 3-4 hyperthyroidism
  - Treat with an initial dose of IV corticosteroid followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
  - Replacement of appropriate hormones may be required as the steroid dose is tapered.

Hepatic:

- For Grade 2 events, monitor liver function tests more frequently until returned to baseline values (consider weekly). Treat with IV or oral corticosteroids
- For Grade 3-4 events, treat with intravenous corticosteroids for 24 to 48 hours.
- When symptoms of Grade 2 events improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks (Grade 3-4 events may require permanent discontinuation).

Renal Failure or Nephritis:

- For Grade 2 events, treat with oral systemic corticosteroids.

- For Grade 3-4 events, treat with intravenous systemic corticosteroids.
- When symptoms of Grade 2 events improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks (Grade 3-4 events may require permanent discontinuation).

Management of Infusion Reactions: Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion.

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.

7.2.4 Concomitant treatments with cytarabine anthracycline chemotherapy

During the induction cycles, G- and GM-CSF may be used only in case of prolonged neutropenia or neutropenic sepsis if non-blastic aplasia is obtained; there will be no such constraints in subsequent cycles. Use of growth factors during consolidation and maintenance is allowed but discouraged.

Antibiotics and antifungals can be given according to institutional rules. For other drugs, refer to individual product US package inserts. Patients should receive all necessary supportive care, including blood product transfusions, erythropoietin, and pain medications.

7.2.5 Tumor Lysis Syndrome Monitoring and Prophylaxis

Patients with a low risk of developing tumor lysis syndrome (TLS) should be monitored for development of TLS and complications; normal hydration and no prophylaxis for hyperuricemia should be given except in cases of signs of metabolic changes, bulky and/or advanced disease and/or high proliferative disease, in which case allopurinol should be added.

Patients with an intermediate risk of developing TLS should be monitored for TLS and complications, administered increased hydration (3 L/m^2 per day) and administered allopurinol (100–300 mg, by mouth [PO], every 8 hours [Q8H], daily) without the need for alkalinization.

In patients with high risk of developing TLS, frequent monitoring should be performed, increased hydration (3 L/m^2 per day), unless evidence of renal insufficiency and oliguria, and rasburicase (0.1–0.2 mg/kg) for one dose and repeated only if clinically necessary. In patients with a prior history of glucose-6-phosphate dehydrogenase, rasburicase is contraindicated and allopurinol should be utilized instead ofrasburicase.

Furthermore, management of hyperkalemia and/or hyperphosphatemia should be managed as per institutional routine. Lastly, patients who develop laboratory TLS who were originally classified as either low or intermediate risk, should receive rasburicase unless clinically contraindicated.

7.3 Duration of Therapy

In the absence of treatment delays due to AE(s), treatment may continue for up to 2 years of

maintenance therapy from first day 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 Protocol Principal Investigator must be consulted.
  - Bone marrow and peripheral blood 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.

7.4 Duration of Follow-Up

Patients will be followed up for survival and remission status for at least every 6 months for up to 5 years after the randomization or until death, whichever occurs first. Patients will be monitored for AE of Special Interest(s) post-transplant for the first 100 days, particularly GVHD and VOD, in both arms. We additionally recommend for the investigators that the patient not receive pembrolizumab within 30 days of receipt of transplant. Patients removed from study for unacceptable AE(s) will be followed until resolution or stabilization of the AE.

7.5 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 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 maybe allowed to continue on study on case-by-case basis based on consultation with study chair and CTEP.

7.6 Contraception and Pregnancy

7.6.1 Contraception

Pembrolizumab (MK-3475) may have adverse effects on a fetus in utero. Furthermore, it is not known if pembrolizumab (MK- 3475) has transient adverse effects on the composition of sperm.

Based on the mechanism of action and findings from animal reproduction studies, fetal harm may occur if cytarabine is administered during pregnancy. Risk to the fetus is decreased if treatment can be avoided during the first trimester; however, females of reproductive potential should avoid becoming pregnant during treatment and be advised of the potential risks.

Idarubicin: Adverse events were observed in animal reproduction studies. Women of reproductive potential avoid pregnancy during treatment.

Daunorubicin: Adverse events have been observed in animal reproduction studies.

Daunorubicin crosses the placenta. Women of reproductive potential should avoid pregnancy.

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:

1. 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
2. Have had a hysterectomy and/or bilateral oophorectomy, or bilateral salpingectomy at least 6 weeks prior to screening.
   OR
3. 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:

1. Practice abstinence† from heterosexual activity.
   OR
2. Use (or have their partner use) adequate 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.

7.6.2 Use in Pregnancy

If a patient inadvertently becomes pregnant while on treatment with Pembrolizumab (MK-3475), 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.

7.6.3 Use in Nursing Women

It is unknown whether Pembrolizumab (MK-3475) 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.

7.7 Treatment Up to 2 Years

Treatment with Pembrolizumab (MK-3475) monotherapy will continue for up to 2 years, 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.

8 DOSING DELAYS/DOSE MODIFICATIONS

Dosing delays and dose modifications for 7+3 (along with added needed 5+2) and HiDAC therapy should follow institutional guidelines and/or treating threating physician's opinion.

8.1 Pembrolizumab Dose Modification and Supportive Care Guidelines for Drug-Related Adverse Events (revised 1-10-2023)

8.1.1 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 1.1.3.

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.

8.1.2 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.

8.1.3 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.

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	Monitor participants for signs and symptoms of enterocolitis (*i.e.*, diarrhea, abdominal pain, blood, or mucus in stool with

	Recurrent Grade 3 or Grade 4	Permanently discontinue	Patients who do not respond to corticosteroids should be seen by a gastroenterologist for confirmation of the diagnosis and consideration of secondary immune suppression	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.
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).

Event	Management
Immune-mediated myelitis, Grade 1	• Continue pembrolizumab unless symptoms worsen or do not improve. • Investigate etiology and refer patient to a neurologist.
Immune-mediated myelitis, Grade 2	• Permanently discontinue pembrolizumab. • Investigate etiology and refer patient to a neurologist. • Rule out infection. • Initiate treatment with corticosteroids equivalent to 1-2 mg/kg/day oral prednisone.
Immune-mediated myelitis, Grade 3 or 4	• Permanently discontinue pembrolizumab. • Refer patient to a neurologist. • Initiate treatment as per institutional guidelines.

Event	Management
Immune-mediated meningoencephalitis, all grades	• Permanently discontinue pembrolizumab. [a] • Refer patient to neurologist. • 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. • If event resolves to Grade 1 or better, taper corticosteroids over ≥1 month.

[a] 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).

8.1.4 Non-immune-related Adverse Events

Additionally, Pembrolizumab (MK-3475) will be withheld for other drug-related grade 4 hematologic toxicities (except for during the induction and consolidation phase), non-hematological toxicity ≥ grade 3 including laboratory abnormalities that are judged as possibly or potentially related to Pembrolizumab (MK-3475), and severe or life-threatening AEs. The table below includes dose modification guidelines for other toxicities that do not appear to be irAEs.

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

Toxicity	Grade	Hold Treatment (Y/N)	Timing for restarting treatment	Dose/Schedule for restarting treatment	Discontinue Subject
	4	Yes	N/A	N/A	Subject must be discontinued
*Note: As severe cytopenias during induction and consolidation phase of AML therapy are expected due to Intensive chemotherapy and the underlying disease, Pembrolizumab (MK-3475) will not be held for hematologic toxicity during these parts of the protocol therapy except in situations where it is felt causing or contributing to the hematologic toxicity.					

In case toxicity does not resolve to grade 0-1 within 12 weeks after last infusion, protocol treatment should be discontinued. With Protocol Principal Investigator 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.

8.2 Commercial Agents

Below are proposed guidelines for adjustments, but investigators should determine the most appropriate course of action based on the specifics of the situation and circumstances for each individual patient.

8.2.1 Cytarabine

Hepatic Impairment

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

Renal Impairment

CrCl >60 mL/min: no dosage adjustment needed.
CrCl ≤60 mL/min: increased risk of cerebellar and cerebral toxicity with high dose regimens. a recommended dosage adjustment is as follows: for a serum creatinine of 1.5–1.9 mg/dL or increase of serum creatinine from baseline of 0.5–1.2 mg/dL during treatment, reduce the dose of cytarabine to 1 g/m^2/dose. for a serum creatinine ≥2 mg/dL or if the change in baseline serum creatinine was >1.2 mg/dL, reduce the dose of cytarabine to 100 mg/m^2/day continuous IV infusion.

8.2.2 Daunorubicin

Hepatic Impairment

Total bilirubin 1.2–3 mg/dL: **REDUCE RECOMMENDED DOSE BY50%.**
Total bilirubin >3–5 mg/dL: reduce recommended dose by 75%.
Total bilirubin >5 mg/dL: omit dose.

Renal Impairment

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

8.2.3 Idarubicin

Hepatic Impairment

Total bilirubin 2.5–5 mg/dL: reduce recommended dose by 50%.
Total bilirubin >5 mg/dL: do not administer.

Renal Impairment

Dosage adjustment is recommended in patients with a serum creatinine greater than 2.5 mg/dL, but no quantitative recommendations are available.

9 PHARMACEUTICAL 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.

9.1 CTEP IND Agent

9.1.1 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).

9.1.2 Agent Ordering and Agent Accountability

9.1.2.1 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 AURORA PMB Online Agent Order Processing application. Access to AURORA 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 AURORA website for specific policies and guidelines related to agent management; https://ctepcore.nci.nih.gov/aurora.

9.1.2.2 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.

9.1.2.3 Material Safety Data Sheets –
The current versions of the material safety data sheets (MSDS or SDS) for PMB-distributed agents will be accessible to site investigators and research staff through the PMB AURORA application. Questions about MSDS access may be directed to the PMB at PMBAfterHours@mail.nih.gov or by using the dialog function in AURORA to communicate with PMB staff.

9.1.2.4 Product Quality Complaint (PQC)
A product quality complaint is defined as any suspicion of a product defect related to a potential quality issue during manufacturing, packaging, release testing, stability monitoring, dose preparation, storage or distribution of the product, or delivery system. Not all PQCs involve a study subject. Lot or batch numbers are of high significance and need to be provided where and when possible. PQC must be reported to the PMB as soon as the PQC is identified. Report PQC to PMB at PMBAfterHours@mail.nih.gov or by using the dialog function in AURORA to communicate with PMB staff.

9.1.3 Investigator Brochure Availability

The current versions of the IBs for the agents will be accessible to site investigators and research staff through the AURORA PMB application. Access to AURORA 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.

9.1.4 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 AURORA application: https://ctepcore.nci.nih.gov/aurora.
- 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)

9.2 Commercial Agents

9.2.1. Cytarabine NSC # 63878

Other Names : ara-C

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

Classification: antineoplastic

Molecular Weight: 243.217 g/mol

CAS Number: 63878

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

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

Side effects include:

- Cardiovascular: Angina pectoris, chest pain, local thrombophlebitis, pericarditis
- Central nervous system: Aseptic meningitis, cerebral dysfunction, dizziness, headache, neuritis, neurotoxicity, paralysis (intrathecal and IV combination therapy), reversible posterior leukoencephalopathy syndrome
- Dermatologic: Acute generalized exanthematous pustulosis, alopecia, dermal ulcer, ephelis, pruritus, skin rash, urticaria

- Endocrine & metabolic: Hyperuricemia
- Gastrointestinal: Abdominal pain, anal fissure, anorexia, diarrhea, esophageal ulcer, esophagitis, increased serum amylase, increased serum lipase, intestinal necrosis, mucositis, nausea, pancreatitis, sore throat, toxic megacolon, vomiting
- Genitourinary: Urinary retention
- Hematologic & oncologic: Anemia, bone marrow depression, hemorrhage, leukopenia, megaloblastic anemia, neutropenia (onset: 1 to 7 days; nadir [biphasic]: 7 to 9 days a nd at 15 to 24 days; recovery [biphasic]: 9 to 12 days and at 24 to 34 days), reticulocytopenia, thrombocytopenia (onset: 5 days; nadir: 12 to 15 days; recovery 15 to 25 days)
- Hepatic: Hepatic insufficiency, hepatic sinusoidal obstruction syndrome (formerly known as hepatic veno-occlusive disease), increased serum transaminases (acute), jaundice
- Hypersensitivity: Allergic edema, anaphylaxis
- Infection: Sepsis
- Local: Cellulitis at injection site, inflammation at injection site (SC injection), local inflammation (anus), pain at injection site (SC injection)
- Neuromuscular & skeletal: Rhabdomyolysis
- Ophthalmic: Conjunctivitis
- Renal: Renal insufficiency
- Respiratory: Acute respiratory distress, dyspnea, interstitial pneumonitis
- Miscellaneous: Drug toxicity (cytarabine syndrome; chest pain, conjunctivitis, fever, maculopapular rash, malaise, myalgia, ostealgia), fever

Availability: Commercially available.

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

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

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

Route of Administration: Continuous IV, per institutional guidelines.

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

9.2.2 Daunorubicin NSC # 82151

Other Names : Cerubidin

Classification:

antineoplastic **Molecular**

Weight: 527.52 g/mol

CAS Number: 82151

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

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

Side effects include:

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

Availability: Commercially available

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

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

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

Route of Administration: slow IV push over 10 to 15 minutes into the tubing of a rapidly infusing IV solution of 1L D5W or NS or and infuse over 15 to 30 minutes), or as per standard institutional practice.

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

9.2.3 Idarubicin NSC # 256439

Other Names : Idamycin

Classification: antineoplastic

Molecular Weight: 497.494 g/mol

CAS Number: 256439

Mode of Action: Idarubicin inhibits DNA and RNA synthesis by intercalation between DNA base pairs and by steric obstruction. Although the exact mechanism is unclear, it appears that direct binding to DNA (intercalation) and inhibition of DNA repair (topoisomerase II inhibition) result in blockade of DNA and RNA synthesis and fragmentation of DNA.

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

Side effects include:

- >10%:
    - Cardiovascular: Cardiac failure (dose-related), ECG abnormalities (transient; includes atrial premature contractions, S-T wave changes, supraventricular tachycardia, ventricular premature contractions; generally asymptomatic and self-limiting)
    - Central nervous system: Headache
    - Dermatologic: Alopecia (25% to 30%), skin rash (11%), urticaria
    - Gastrointestinal: Vomiting (30% to 60%), gastrointestinal hemorrhage (30%), diarrhea (9% to 22%), stomatitis (11%), nausea
    - Genitourinary: Urine discoloration (darker yellow)
    - Hematologic & oncologic: Anemia (effects are generally less severe with oral dosing), bone marrow suppression (nadir: 10 to 15 days; recovery: 21 to 28 days, primarily leukopenia, effects are generally less severe with oral dosing), thrombocytopenia (effects are generally less severe with oral dosing)
    - Hepatic: Increased serum bilirubin (≤44%), increased serum transaminases (≤44%)
    - Miscellaneous: Radiation recall phenomenon
- 1% to 10%:

- Central nervous system: Peripheral neuropathy, seizure

Availability: Commercially available.

Product Description: Idarubicin hydrochloride for injection, USP is a sterile, semi-synthetic antineoplastic anthracycline for intravenous use. Chemically, idarubicin hydrochloride is 5,12-Naphthacenedione, 9-acetyl-7-[(3-amino-2,3,6-trideoxy-α-L-lyxohexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,9,11-trihydroxyhydrochloride, (7S-cis). Idarubicin is commercially available.

Preparation: 20 mg vials are reconstituted with 20 ml of water. Refer to the package insert for standard preparation instructions.

Storage and Stability: Stored at controlled room temperature, 15° to 30°C (59° to 86°F) and protected from light.

Route of Administration: slow IV push over 10 to 15 minutes into the tubing of a rapidly infusing IV solution of 1L D5W or NS or and infuse over 15 to 30 minutes), or as per standard institutional practice.

Potential Drug Interactions: Refer to drug package insert for additional information.

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

10 STATISTICAL CONSIDERATIONS

10.1 Study Design/Endpoints

This is an open label, randomized, phase 2 study. The study will consist of an induction, consolidation, and maintenance phase of therapy. A total of 50 patients will be included (25 patients in the intervention arm and 25 patients in the control arm).

Induction chemotherapy for AML has been demonstrated to result in MRD negative CR (not just CR) in about 50% of patients treated (Freeman *et al*., 2013). Few large data sets of MRD negative CR exist across different subpopulations. We hypothesize that the addition of MK-3475 (pembrolizumab) to induction chemotherapy will lead to a 25% improvement of MRD negative CR/CRi after induction and one cycle of consolidation therapy compared to 7+3 and one cycle of consolidation chemotherapy alone. We expect a response rate of 75% in the intervention arm and 50% in the control arm for patients completing their respective treatments. Using intention-to-treat analysis, in open label study, with 25 patients in the intervention arm and 25 patients in the control arm and a significance level of 0.1, we have approximately 71% power to detect an MRD-negative CR/CRi response rate difference of 25% using a one-sided Z-test for two proportions.

Early safety schedule change: After the first 6 patients have been enrolled on the experimental 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. We will temporarily hold enrollment in the study if two or more patients of the first evaluable six patients in pembrolizumab (MK-3475) arm develop pembrolizumab-related severe toxicity during evaluability period. The evaluability period includes the first 35 days after pembrolizumab first dose, till hematologic recovery, or till starting consolidation therapy, whichever occurs first, as defined in section 9.5.1. To be considered evaluable for purposes of the early safety rule, the patient must have received at least one pembrolizumab dose. If the true toxicity rate is 50% or greater, this rule will stop the trial with probability at least 0.89. Similarly, if the true toxicity rate is 33%, this stopping rule will end the trial with probability 0.65. In the event this occurs, the study would resume with another 6-patient cohort in which pembrolizumab dosing will start on Day 21 instead of Day 8. The patient has to receive at least one dose of pembrolizumab to be considered evaluable for safety assessment for purposes of early stopping safety rule. If the altered schedule still results in unacceptable toxicity, we will discuss alternative approaches with CTEP. No enrollment may take place until this discussion has occurred and a suitable plan has been developed.

Toxicity analysis: The first six enrolled patients will be assessed for severe toxicity in the pembrolizumab arm. In the event that severe toxicity occurs in 2 or more of the first 6 patients, the study would resume with another 6-patient cohort in which pembrolizumab dosing will start on Day 21 instead of Day 8. If the altered schedule still results in unacceptable toxicity, we will discuss alternative approaches with CTEP. A formal toxicity analysis will be conducted after 25 efficacy-evaluable patients in each study arm.

Follow up: For patients who neither relapse nor die, the DOR will be censored on the date of their last evaluable disease assessment. Median 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 three years from first dose. An analysis of the survival and RFS data will be conducted based on the five-year follow up from date of randomization. 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.

10.1.1 Primary endpoint

MRD-negative CR/CRi response rate at time of count recovery after first cycle of consolidation therapy with chemotherapy + Pembrolizumab (MK-3475) vs. 7+3 and one cycle of consolidation chemotherapy alone.

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

The current state of the art in AML MRD by flow cytometry can detect 0.1% for ~95% of patients and 0.01% for a major subset of those (~40-50%) but is heavily dependent on the immunophenotype of the leukemia and nature of the background population. The sensitivity of the assay varies depending on those two variables, so the threshold at present is not uniformly

0.01% for all patients. Importantly, the primary futility and efficacy analyses will compare MRD negative CR/CRi rates between the two arms, and if the MRD by flow can detect below this limit of 0.01% in only 40% of patients, then 60% of patients might not contribute to study primary endpoint differential effect. Given the limitations of the current technology, an MRD quantified below <0.1% may still be consistent with residual leukemia. We therefore have decided to use this cutoff to distinguish MRD-positive from MRD-negative patients as this threshold was found relevant in most published studies to date and has been included in the official consensus document on minimal residual disease by the European Leukemia Net MRD Working Party. Thus, cutoff levels below 0.1% (*e.g.*, <0.01%) may define patients with particularly good outcome.

10.2 Sample Size/Accrual Rate

A total of 50 patients (25 per arm) are planned to be enrolled in the intent-to-treat analysis, which will evaluate a 25% difference in the rate of MRD-negative CR/CRi with ~71% power and an overall, one-sided alpha level of 0.1. All patients being randomized and starting induction therapy should be included in the primary analysis.

PLANNED ENROLLMENT REPORT

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	0	0	0	0	**0**
Asian	1	1	0	0	**2**
Native Hawaiian or Other Pacific Islander	1	1	0	0	**2**
Black or African American	1	1	1	1	**4**
White	15	20	1	2	**38**
More Than One Race	1	1	1	1	**4**
Total	**19**	**24**	**3**	**4**	**50**

10.3 Stratification Factors

Planned stratification factors include age (younger than 65 *vs*. 65 and older), cytogenetics by FISH or metaphase karyotype (presence *vs.* absence of core binding factor inversions and translocations), and t-AML or AML arising from prior/antecedent MDS (yes/no). Dose escalation and MTD determination are not parts of this study.

10.4 Analysis of Secondary and Exploratory Endpoints

10.4.1 Secondary Endpoints

10.4.1.1 Efficacy Endpoints:

- Assess the rate of CR/CRi as defined per the ELN 2017 (see Appendix D) response criteria at time of count recovery after induction therapy with chemotherapy + Pembrolizumab (MK-3475).
- Assess MRD negativity at Day 14.
- Assess the rate of MRD-negative CR at end of induction therapy.
- Assess the rate of MRD-negative CR at end of consolidation.
- Assess rates of complete remission with partial recovery count (CRh) and Hematologic improvement (HI) to red blood cells and platelets.
- Assess the percentage of patients with MRD-CR at time of count recovery after first cycle of consolidation therapy using a MRD cut-off of 0.01%.
- Assess EFS, RFS, calculated as the time from initial treatment to either disease relapse or death.
- Assess the DOR (defined as the time from first CR to the date of the first documented relapse or death, whichever occurs first) and OS.

10.4.1.2 Safety Endpoints:

- Assess safety endpoints including proportion of patients who develop severe toxicity.

10.4.2 Exploratory Endpoints

10.4.2.1 MRD assessment by DS as an exploratory biomarker

All diagnostic and remission samples will undergo DNA extraction at the Fred Hutchinson

Cancer Research Center. 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: ~1 μg 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.

10.4.2.1.1 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.

10.4.2.1.2 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 ($\dot{\rho}$) 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.

10.4.2.2 Assessment of immune cell subsets and its correlation with the response to combine chemotherapy and anti PD-1 directed therapy

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 mass cytometry (CyTOF). These techniques will provide information about the architecture of the tumor microenvironment (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). 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).

We will assess these immune cell markers at different time points of therapy: At baseline (prior

to application of 7+3 chemotherapy), at the end of consolidation, after 6 months and 1 year of treatment, and at relapse. We also plan to measure CD47 levels on leukemic blasts prior to and after chemotherapy and pembrolizumab 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.

10.4.2.3 PD-1 and PD-L1 expression

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 2×2 table of frequencies. The dependent variable will be defined as response (yes *vs.* 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.

We will assess immune cell markers at different time points of therapy: at baseline (prior to application of 7+3 chemotherapy), at the end of consolidation, after 6 months and 1 year of treatment, and at relapse. We also plan to measure CD47 levels on leukemic blasts prior to and after chemotherapy and Pembrolizumab (MK-3475) administration 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}, TCM/TEMRA, TEM/TEMRA, 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.

10.4.2.4 Olink Assay

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.

10.4.2.5 WES

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.

10.4.2.6 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.

10.4.2.7 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.

10.4.2.8 Gut microbiome

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, 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. 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 modulates 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.

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 first consolidation cycle will also be explored.

Exploratory outcomes:
Correlation between microbial/metabolome changes with immune-checkpoint expression and kinetics of immune cell subset recovery and programming in the standard of care and experimental arm will be evaluated. As a marker of mucosal immunity, changes in immune cell content within stool samples in patients that experience colitis or GI-GVHD will be compared to suitable controls.

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, RNA seq/TCRseq, 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 correction (Benjamini *et al.,* 1995) to control for false discovery rate.

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-consolidation cycle1 and 4, every 3 months during maintenance, one year, and end of treatment when applicable. Landmark analysis (Benjamini *et al.,* 1995, Dafni *et al.,* 2011) or joint modeling (Huang *et al.,* 2016) 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, intensive chemo + pembro vs. intensive chemo only.
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 sum / 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 (Benjamini *et al.,* 1995).

10.5 For phase 2 protocols only: Reporting and Exclusions

10.5.1 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. The evaluability period includes the first 35 days after pembrolizumab first dose, till hematologic recovery, or till starting consolidation therapy, whichever occurs first.

10.5.1.1 Any grade 3 or 4 non-hematologic toxicity, that is deemed related to Pembrolizumab (MK-3475), with the following exceptions: A) Transient laboratory abnormalities that can be treated or resolve to grade 2 or less within 7 days. 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.

10.5.1.2 Grade 4 thrombocytopenia or neutropenia associated with treatment-associated 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, they will not be used to define unacceptable toxicity except if associated with prolonged treatment-associated aplasia AND not associated with active disease.

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

10.5.2 Evaluation of Response

All patients who were randomized 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 accepted ELN2017 response categories (see Appendix D). [Note: By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database.]

All patients who were randomized (with the possible exception of those who received no study medication) should be included in the main analysis of the response rate. Patients who do not achieve CR/CRi after induction and first consolidation will be considered to have a treatment failure. Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate.

All conclusions should be based on all eligible patients. 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 sub-analyses 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.

10.6 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.

11 ADVERSE EVENTS: LIST AND REPORTINGREQUIREMENTS

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.

11.1 Comprehensive Adverse Events and Potential Risks List (CAEPR)

11.1.1 CAEPRs for CTEP IND Agent

11.1.1.1 Comprehensive Adverse Events and Potential Risks list (CAEPR) for MK-3475 (pembrolizumab, 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.

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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	

Adverse Events with Possible Relationship to Pembrolizumab (MK-3475) (CTCAE 5.0 Term) [n= 3793]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
		(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]		
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		

Adverse Events with Possible Relationship to Pembrolizumab (MK-3475) (CTCAE 5.0 Term) [n= 3793]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
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			
		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 -	

Adverse Events with Possible Relationship to Pembrolizumab (MK-3475) (CTCAE 5.0 Term) [n= 3793]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
		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]	
		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.

11.1.2 Common Adverse Events for Commercial Agents

11.1.2.1 Cytarabine

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

Headache

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

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

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

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

11.1.2.2 Daunorubicin

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

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

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

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

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

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

11.1.2.3 Idarubicin

Common AEs for idarubicin include:

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

Pain along the site where the medication was given.

Low blood counts
Urine may appear red, red-brown, orange or pink from the color of the medication
Nausea or vomiting.
Mouth sores.
Hair loss on the scalp or elsewhere on the body
Diarrhea/abdominal cramps

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

Fever
Headache
Nail thickening, nail banding
Discoloration of the skin or nails.
Darkening of the skin where previous radiation treatment has been given. (radiation recall).
Hand -foot syndrome (Palmar-plantar erythrodysesthesia or PPE)
Loss of fertility
Heart rhythm abnormalities
Increases in blood tests measuring liver function.

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

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

Immune check point blockade studies have demonstrated the feasibility and safety of combining immunotherapy with intensive chemo induction feasible and safe in younger AML patients (Ravandi *et al*., 2019). 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 1.5 g/m^2, 24-hour infusion of cytarabine daily on Days 1–4 (three days only for patients >60 years), alongside 12 mg/m^2 daily on Days 1–3 of idarubicin. Nivolumab was given on Day 24 at a dose of 3 mg/kg which was repeated every two weeks for a year in responders. AEs are detailed in the table below.

AEs 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.

11.2 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.

  - **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.

11.3 Expedited Adverse Event Reporting

11.3.1 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.

11.3.2 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.

11.3.3 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 <u>both</u> routine and expedited reporting, regardless of causality as long as the death occurred within 30 days after the last administration of the investigational agent. Attribution to treatment or other cause must be provided.

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

Late Phase 2 and Phase 3 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 **<u>MUST</u>** immediately report to the sponsor (NCI) **<u>ANY</u>** 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 **<u>ANY</u>** 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).

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

Grade 1-3 Timeframes	Grade 4-5 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.

<u>Expedited AE reporting timeframes are defined as:</u>

- "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 4-5 SAEs
- Within 10 calendar days for Grade 1-3 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

11.4 Routine Adverse Event Reporting

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

11.5 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.

11.6 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.*, 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.

11.7 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.

12. STUDY CALENDAR

Screening evaluations are to be conducted within 21 days prior to start of protocol therapy. Blood tests will be taken at every clinic visit for 1 year (if applicable, drawn when routine blood samples are taken). In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

			Induction Phase										Consolidation Phase[a]								Maintenance / Observation Phase £	After 6 Months of Treatment	After 1 Year of Treatment	End of Maintenance / Observation[b]	Post-treatment Follow-Up[c]
													Cycle 1				Cycles 2 [a], 3, 4								
	Screening[y]	D1	D 2	D 3	D 4	D 5	D 6	D7	D 8	D9	D 14	D 28	D 1	D 3	D 5	D 28	D 1	D 3	D 5	D 28					
Cytarabine[d]		X	X	X	X	X	X	X			X[e]		X	X	X		X	X	X						
Daunorubicin or Idarubicin[d]		X	X	X							X[e]														
Pembrolizumab (MK-3475) [f]			Every 3 weeks starting on Day 8 of the Induction phase																						
Informed Consent	X																								
Eligibility Criteria	X																								
Medical History, Demographics	X																								
Prior and Concomitant Medication Review		X ---------- X																							
Survival Status																									X
EKG[w]	X																								
Physical Exam	X	X									X	X[n]				X				X	X			X	X
Vital signs	X	X									X	X[n]				X				X	X			X	X
Height	X																								
Weight	X																								
Performance Status[g]	X											X[n]				X				X	X			X	
Adverse event evaluation			X ---------- X																						
Comprehensive Chemistry Panel[h,v]	X	X	X	X	X	X	X	X	X[u]	X[u]	X	X[n]	X			X	X			X	X[v]			X	

			Induction Phase										Consolidation Phase[a]								Maintenance / Observation Phase £	After 6 Months of Treatment (+/- 14 days)	After 1 Year of Treatment (+/- 14 days)	End of Maintenance / Observation[b]	Post-treatment Follow-Up[c]
													Cycle 1				Cycles 2 [a], 3, 4								
	Screening [y]	D1	D 2	D 3	D 4	D 5	D 6	D7	D 8	D9	D 14	D 28	D 1	D 3	D 5	D 28 β	D 1	D 3	D 5	D 28 β					
CBC with Differential[i,v]	X	X	X	X	X	X	X	X	X	X	X	X[n]	X			X	X			X	X[v]			X	X
Pregnancy Test[j] – Urine or Serum β-HCG	X																								
Urinalysis	X	X									X	X[n]				X				X	X				X
TSH; T3, T4[k]	X								X			X									X				
PT, PTT, fibrinogen									X	X															
Bone marrow[l] Aspiration	X										X[m]	X[n]				X				X[o]	X[p]	X	X	X[s]	
Bone marrow biopsy	X											X[n]				X				X[o]		X	X	X[s]	
Stool	X															X[z]									
Research Blood Draws	X										X[m]	X[n]				X				X[o]	X[q]	X[r]	X[r]	X[s]	
Skin Punch Biopsy α	X																								
Transthoracic ECHO (TTE) or MUGA	X									X[t]															
CT chest	X[x]																								

a If the patient does not have a CR/CRi before starting this part of the study, the patient should go off study. Patients <65 may complete 3-4 cycles of consolidation therapy. Patients ≥65 years old may complete 1-2 cycles of consolidation therapy.

b End of treatment visit will be immediately following final dose of Pembrolizumab (MK-3475).

c After completing study treatment, clinical follow-up assessments are required every 6 months from date of therapy discontinuation. Common clinical practice should be monitored by institutional practice (*i.e.*, every 3 months though not required by protocol), but assessment results are only required every 6 months (+/- 14 days). 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.

d Cytarabine should be dosed as appropriate for each phase of the study. Daunorubicin (60 mg/m^2/day) or Idarubicin (12 mg/m^2/day)

e If patient has residual leukemia at the time of bone marrow aspiration, the patient may receive a 5+2 administration of these drugs. Adjust anthracycline dose as per institutional standards for Re- Induction.

f Only for patients randomized to Arm B at 200 mg. Pembrolizumab (MK-3475) will be administered on Day 8 of induction and then Q3 weeks (+/- 3 days). Do not administer pembrolizumab concurrently with HIDAC chemotherapy Days 1-5 of each consolidation cycle. For consideration of timing of pembrolizumab, allow at least 3 hours after completion of chemotherapy prior to pembrolizumab administration. Administration window for pembrolizumab may be extended to allow for this timing as necessary. Each cycle of Maintenance is 21 days with pembrolizumab given on day 1 of each cycle up to 2 years.

g Note: Performance status evaluations are based on a 4-week cycle. At minimum, performance status should be evaluated at the beginning of every cycle.

h Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.

i 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.

j 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 Arm A and Arm B. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

k. TSH should be performed at baseline and <72 hours prior to each dose of Pembrolizumab (MK-3475). Additional T3 and T4 tests should be performed as clinically indicated, if TSH is abnormal, if symptoms are suggestive of thyroid dysfunction.

l. Bone marrow aspirate is required to be submitted for central MRD testing as outline in Section 5. Submit to Dr. Brent Wood's lab at 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.

m To be collected between Day 14 and Day 21.

n To be collected between Day 28 and Day 42.

o To be collected at the end of consolidation therapy only.

p Bone marrow aspirations every 3 months during maintenance/observation phase are optional and should only be collected when part of standard of care.

q To be collected every 3 months during maintenance phase.

r Blood collection for the immune cell subset analysis and Olink assay only.

s Bone marrow aspiration and blood should be collected for MRD status in patients who have not relapse. For patients who have relapsed, bone marrow core biopsies, bone marrow aspirate, and blood should be collected. Blood collection for cfDNA will be obtained at the same time-points as blood collection for MRD status.

t. Repeat TTE should be performed before 5+2 reinduction.

u PT, PTT, and fibrinogen will be additionally collected on days 8 and 9 (pre- and post-) in the pembrolizumab arm.

v CBC and CMP will be obtained within 3 days of receipt of each pembrolizumab dose in the pembrolizumab arm and every 3 weeks during treatment and every 6 (+/- 1 week) weeks during observation for patients in the control arm.

w ECGs performed after screening will be done as medically indicated.

x CT chest should be considered as a part of screening (optional)

y Screening period is up to 21 days

z At time of Post-Consolidation Cycle 1 bone marrow collection (+/- 3 days)

α Refer to Section 5.1 Summary Table for Specimen Collection and Appendix F Skin Punch Biopsy Verification Form

β (+/- 3) days

£ Observation visits in Maintenance on control arm may be extended to 6 weeks (+/- 1 week).

13 MEASUREMENT OF EFFECT

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

13.1 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with 7+3 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). 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.

13.2 Hematologic Response

13.2.1 **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

13.2.2 **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

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

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

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

13.2.6 **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%)

13.2.7 **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 ≥1.0 × 10^9/L.
- Platelet count ≥100 × 10^9/L.
- Reduced hemoglobin concentration or hematocrit has no bearing on remission status.
- Leukemic blasts must not be present in the peripheral blood.

Bone Marrow Aspirate and Biopsy:

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

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

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

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 is 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 on treatment with stable disease at their discretion if they judge the patient to be deriving clinical benefit on study.

13.2.9 **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.

13.2.9.1 MRD Response

Refer to section

9.1.1

13.3 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.

14 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).

14.1 Study Oversight

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

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

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

14.2 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.

14.2.1 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 ofCRFs.

14.2.2 Responsibility for Data Submission

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

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

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

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

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

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

14.3 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.

14.4 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:

14.4.1 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.

14.4.2 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"):

14.4.2.1 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.

14.4.2.2 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.

14.4.2.3 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 aproval, and commercialization of its own Agent.

14.4.3 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.

14.4.4 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.

14.4.5 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.

14.4.6 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.

14.5 Genomic Data Sharing Plan

The investigators and statistician and/or bioinformaticians for a study will have access to all data on mutations and variants stored in the Theradex Data Base and the GDC. This information will be sequestered from access throughout the study until it is analyzed for purposes of reporting and publishing of the study results. As specified in the CRADA for the agents used in the clinical study, the pharmaceutical collaborator will have at least 6 months, longer if needed for a regulatory filing, to review the data and or receive copies of the data once the study is completed and analyzed, or sooner, if specified for purposes of generating Intellectual Property. Once these timeframes have been exceeded, the data will be available through a Data Access Committee (DAC) in the GDC following NCI and Collaborator review of the proposals.

14.6 Incidental/Secondary Findings Disclosure Procedure

Given the potential clinical implications conferred by detecting a germline and/or somatic mutation in one of the proven cancer susceptibility genes, this protocol will use the following disclosure procedure, consistent with the recommendations of the American College of Medical and Genomics (ACMG) (Green *et al*., 2013 and Kalia *et al.,* 2016):

The NCI Molecular Characterization Laboratory will review the mutations/variants once at the time of initial specimen evaluation according to the most recent version of the ACMG guidance on variants. The NCI Molecular Characterization Laboratory will not re-review all specimens received if a new version of the ACMG guidance is published after the initial review.

For each participant with a pathogenic or likely pathogenic germline and/or somatic variant detected in the WES of blood (as defined in the ACMG guidance), the NCI Molecular Characterization Laboratory will report to the Program Director or Scientific Officer the UPID and variant(s) identified. The Program Director or Scientific Officer will contact Theradex to obtain the name of the protocol, investigator treating the patient, and the Principal Investigator of the grant. The treating physician will be contacted by phone and in writing to ask the patient whether he or she is interested in learning more about the finding.

If the patient wants to know more, the physician should contact the Program Director for more information about the mutation/variant. The treating physician and a medical genetics counselor should meet with the patient to discuss the importance and meaning of the finding, but not the finding itself, and notify the patient that this research finding must be confirmed by Sanger sequencing at the patient's/patient insurer's expense in a Clinical Laboratory Improvement Amendments (CLIA)-approved laboratory. The treating physician and genetic counselor should inform the patient of the confirmed result and its meaning and significance to the patient. If desired, the patient may elect to undergo genetic counseling and confirmatory CLIA-approved clinical testing on his or her own. Neither the research laboratory nor the National Cancer Institute will be responsible for the costs incurred for any confirmatory genetic testing or counseling.

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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		
Female	≤62 (≤0.7)	$GFR = 166 \times (SCr/0.7)^{-0.329} \times (0.993)^{Age}$
	>62 (>0.7)	$GFR = 166 \times (SCr/0.7)^{-1.209} \times (0.993)^{Age}$
Male	≤80 (≤0.9)	$GFR = 163 \times (SCr/0.9)^{-0.411} \times (0.993)^{Age}$
	>80 (>0.9)	$GFR = 163 \times (SCr/0.9)^{-1.209} \times (0.993)^{Age}$
White or other		
Female	≤62 (≤0.7)	$GFR = 144 \times (SCr/0.7)^{-0.329} \times (0.993)^{Age}$
	>62 (>0.7)	$GFR = 144 \times (SCr/0.7)^{-1.209} \times (0.993)^{Age}$
Male	≤80 (≤0.9)	$GFR = 141 \times (SCr/0.9)^{-0.411} \times (0.993)^{Age}$
	>80 (>0.9)	$GFR = 141 \times (SCr/0.9)^{-1.209} \times (0.993)^{Age}$

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

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

$175 \times SCr^{-1.154} \times age^{-0.203} \times 0.742$ (if female) $\times$ 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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2. Levey, A.S., J. Coresh, T. Greene, *et al*. (2006). Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Intern Med*. 145:247-254.
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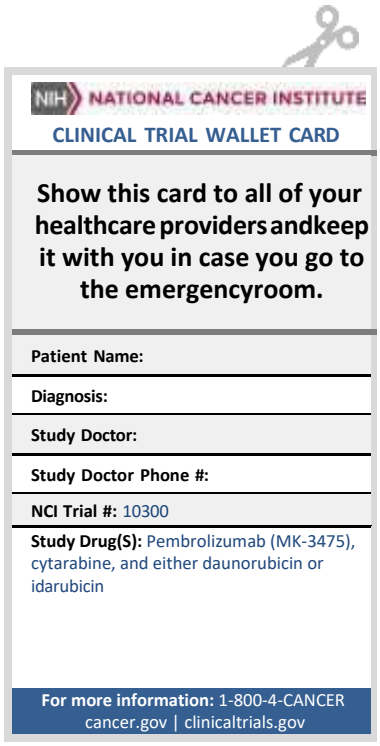
APPENDIX C PATIENT CLINICAL TRIAL WALLET CARD



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 #: 10300

Study Drug(S): Pembrolizumab (MK-3475), cytarabine, and either daunorubicin or idarubicin

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

CR with incomplete hematologic recovery (CRh): All CR criteria with neutrophil count (>0.5 × 10^9/L [1000/μL]) and platelet count (>50 × 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] nontransfused)
• >50% increase in peripheral blasts (WBC × % blasts) to >25 × 10^9/L (>25 000/μL) (in the absence of differentiation syndrome)
• New extramedullary disease Some protocols may allow transient addition of hydroxyurea to lower blast counts
"Progressive disease" is usually accompanied by a decline in ANC and platelets and increased transfusion requirement and decline in performance status or increase in symptoms

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 BONE MARROW CORE BIOPSY SAMPLES

- Tissue ***must be fixed*** in neutral-buffered formalin (no acid-basedproducts).
- ***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

APPENDIX F SKIN PUNCH BIOPSY VERIFICATION FORM

Please have the Clinician* responsible for signing out this patient's case complete the following:

ETCTN Universal Patient ID: _____

ETCTN Patient Study ID: _____

Date of Procedure (mm/dd/yyyy): _____

Tissue Type (circle one): Normal Skin Other:_____

Time point (circle one): Baseline Other:_____

Site Tissue Taken From: _____

I agree that this tissue may be released for research purposes only and that the release of this tissue will not have any impact on the patient's care.

_____ _____
Clinician Signature Date

_____
Clinician Printed Name

*Note: For the purposes of this form, Clinician could include the Nurse Practitioner, Registered Nurse, Pathologist, Radiologist, Interventional Radiologist, Surgeon, Oncologist, Internist, or other medical professional responsible for the patient's care.

Version: 1
Effective Date: 9/2022