Phase II Trial of Pembrolizumab after High Dose Cytarabine in Relapsed/Refractory Acute
Myeloid Leukemia

Joshua F. Zeidner1, 2, Benjamin G. Vincent1, 2, 3, 4, Anastasia Ivanova5, Dominic Moore5, Karen P. McKinnon1, 3, Alec D. Wilkinson1, Rupkatha Mukhopadhyay6, Francesco Mazzioita6, 7, Hanna A. Knaus6, Matthew C. Foster1, 2, Catherine C. Coombs1, 2, Katarzyna Jamieson1, 2, Hendrik Van Deventer1, 2, Jonathan A. Webster6, 8, Gabrielle T. Prince6, 8, Amy E. DeZern6, 8, B. Douglas Smith6, 8, Mark J. Levis6, 8, Nathan D. Montgomery1, 9, Leo Luznik6, 8, Jonathan S. Serody1, 2, 3, 4*, Ivana Gojo6, 7*

1- University of North Carolina School of Medicine, Lineberger Comprehensive Cancer Center, Chapel Hill, NC 27599
2- Division of Hematology, Department of Medicine, University of North Carolina School of Medicine, Chapel Hill, NC 27599
3- University of North Carolina Department of Microbiology and Immunology, Chapel Hill, NC 27599
4- Program in Computational Medicine, University of North Carolina at Chapel Hill, Chapel Hill NC 27599
5- University of North Carolina School of Medicine, Department of Biostatistics, Chapel Hill, NC 27599
6- Johns Hopkins School of Medicine, Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD 21231
7- University of Siena, Department of Medical Biotechnologies, Siena, Italy
8- Department of Oncology, Division of Hematological Malignancies, Johns Hopkins School of Medicine, Baltimore, MD 21231
9- Department of Pathology and Laboratory Medicine, University of North Carolina School of Medicine, Chapel Hill, NC 27599

*- Contributed equally

Running Title: HiDAC + Pembrolizumab in R/R AML

Word Count: 5,636

Abstract Word Count: 154

Total Number of Tables, Figures- 7, 53 References

Disclosure of Potential Conflicts of Interests:

J.F.Z. has received consultancy fees from AbbVie for serving on an Independent Review Committee, AsystBio Laboratories, and Takeda; received honoraria from advisory boards from Agios/Servier, Bristol Myers Squibb/Celgene, Daiichi Sankyo, Genentech, Gilead, Pfizer, Shattuck Labs, and Takeda; received research funding from Arog, Astex, Gilead/Forty Seven, Merck, Sumitomo Dainippon Pharma, and Takeda.

B.G.V. holds equity and has received consulting fees from GeneCentric Therapeutics.

M.C.F. has served as a consultant for Daiichi Sankyo; has received research funding from Bellicum, Bristol Myers Squibb/Celgene, and Macrogenics.
C.C.C. has served on steering committee for LOXO and AbbVie; received consulting fees from AbbVie; received honoraria from LOXO, AbbVie, Astra Zeneca, MEI Pharma, Novartis and Octapharma.

A.D. has received honoraria from AbbVie, Bristol Myers Squibb and Novartis.

L.L. holds a Patent with WindMiL Therapeutics; Grant/Research/Clinical Trial Support: Genentech; Consultant/Advisory Boards: Gilead Sciences, Rubius Therapeutics, PrecisionBiosciences, Talaris Therapeutics

J.S.S. has received research funding from Merck Sharpe & Dohme, GlasxoSmithKline, and Carisma Therapeutics; has patents pending on use of STING agonists and CAR T cells.

I.G. has served as a consultant for AbbVie, Amgen Inc., Bristol Myers Squibb, Certara, Jazz, Novartis, and Ono Pharma; has received research funding from Amphivena, Amgen Inc., Celgene, Genentech, Gilead, Juno, and Merck.

All other authors declare no conflicts of interest.

**Authorship Contributions:**

J.F.Z. and I.G. contributed to study design, enrolled patients, collected and interpreted the data, and wrote the manuscript. B.G.V, L.L and J.S.S. contributed to study design, collected and interpreted the data, and wrote the manuscript. A.I. contributed to study design and interpreted the data. D.M. interpreted the data. K.M., A.W., R.M., and F.M. interpreted the data and wrote the manuscript. H.A.K. and N.D.M. interpreted the data. M.C.F., C.C.C., K.J., H.V.D., J.W., G.P., A.D., B.D.S., and M.L. enrolled patients. All authors provided critical review and final approval of the manuscript.

**Corresponding Author:**

Joshua Zeidner, MD

Associate Professor of Medicine

170 Manning Drive, Houpt Building

CB #7305

Chapel Hill, NC 27599

Joshua_Zeidner@med.unc.edu

(919) 962-5164

Fax: (919) 966-6735
Abstract:
Immune suppression, exhaustion and senescence are frequently seen throughout disease progression in AML. We conducted a phase II study of high-dose cytarabine followed by pembrolizumab 200 mg IV on day 14 to examine whether PD-1 inhibition improves clinical responses in relapsed/refractory (R/R) AML. Overall responders could receive pembrolizumab maintenance up to two years. Among thirty-seven patients enrolled, the overall response rate, composite complete remission rate (CRc; primary endpoint), and median overall survival (OS) were 46%, 38% and 11.1 months, respectively. Patients with refractory/early relapse and those receiving treatment as first salvage had encouraging outcomes (median OS 13.2 and 11.3 months, respectively). Grade ≥3 immune-related adverse events were rare (14%) and self-limiting. Patients who achieved CRc had a higher frequency of progenitor exhausted CD8+ T-cells expressing TCF-1 in the bone marrow prior to treatment. A multifaceted correlative approach of genomic, transcriptomic and immunophenotypic profiling offer insights on molecular correlates of response and resistance to pembrolizumab.

Significance:
Immune checkpoint blockade with pembrolizumab was tolerable and feasible after high-dose cytarabine in relapsed/refractory AML with encouraging clinical activity, particularly in refractory AML and those receiving treatment as first salvage regimen. Further study of pembrolizumab and other immune checkpoint blockade strategies after cytotoxic chemotherapy is warranted in AML.
Introduction:

Despite therapeutic advancements in the management of patients with AML over the last several years, outcomes remain dismal for those with relapsed/refractory (R/R) disease. The advent of targeted therapeutic approaches has changed the treatment landscape providing salvage options for a subset of patients. Nonetheless, complete remission (CR) rates of approximately 20-30% and median overall survival (OS) of 8-9 months in R/R AML patients with FLT3, IDH1 and IDH2 mutations treated with gilteritinib(1), ivosidenib(2), and enasidenib(3), respectively, underscore the poor outcomes in this patient population even with targeted therapies.

The majority of R/R AML patients do not have targeted treatment options. Intensive salvage chemotherapy regimens including high dose cytarabine (HiDAC) are generally employed for younger, fit patients, though there is currently no standard-of-care or salvage chemotherapy regimen that has consistently been shown to improve outcomes in R/R AML.(4-6) Multiple prognostic factors such as duration of CR, age, cytogenetics, and previous history of allogeneic stem cell transplantation (alloSCT) can discriminate 1-year OS rates from 16 to 70% based on overall risk status at the time of AML relapse.(7) However, in a pooled analysis of R/R AML patients treated with first salvage chemotherapy, CR and median OS rates were approximately 14% and 6.3 months, respectively, and outcomes are worse in those receiving second or third salvage chemotherapy.(8) Thus, there is a large unmet need to develop novel therapeutic approaches in this patient population.

AML patients carry innate and adaptive immune aberrations at diagnosis that lead to immune suppression, exhaustion and senescence.(9-11) Multiple studies have shown that up-regulation of inhibitory receptors (IRs), such as the programmed-death 1 (PD-1)/PD-L1 axis, plays a role in immune evasion by leukemic cells.(11-17) PD-1 is expressed on the surface of activated T cells, B cells and natural killer cells. When bound by its ligands, PD-1 stimulation leads to suppression
of T cell activation and inhibition of T cell responses. Pre-clinical models have shown that PD-1 expressing CD8\(^+\) T cells and regulatory T cells (T\(_{\text{regs}}\)) accumulate during AML progression leading to T cell exhaustion which can be restored by T\(_{\text{reg}}\) depletion followed by PD-1/PD-L1 blockade.\(^{(12)}\) Further, PD-1 knockout mice have less leukemia burden and improved OS.\(^{(12)}\) Up-regulation of PD-L1 on leukemic blasts is more frequently observed at relapse than at diagnosis and is associated with poor prognosis.\(^{(13, 18)}\) Similarly, the frequency of T cells co-expressing multiple IRs increases with disease progression.\(^{(11, 17)}\) Reversibility of the phenotypic and transcriptional signatures of CD8\(^+\) T cells in AML patients who achieve CR suggests that T cell exhaustion, an important feature of R/R AML, may be susceptible to therapeutic intervention such as PD-1/PD-L1 axis blockade.\(^{(11)}\)

Monoclonal antibody blockade of IRs, most specifically PD-1, has led to a paradigm shift in the management of cancer with a multitude of FDA-approvals and breakthrough therapies. However, there is a dearth of clinical data with these agents in combination with cytotoxic chemotherapy in R/R AML. We designed a phase II study of HiDAC followed by pembrolizumab, a human IgG4 monoclonal antibody targeting PD-1, in R/R AML, the first study investigating the combination of immune checkpoint blockade (ICB) with cytotoxic salvage chemotherapy. Pembrolizumab was administered on day 14 after completion of HiDAC during a time period of expected heightened inflammation and early onset of lymphocyte recovery.\(^{(10)}\) This clinical-translational study was designed with the hypothesis that pembrolizumab would augment anti-leukemia T cell responses and improve the clinical activity of HiDAC in R/R AML. Additionally, we used flow cytometry and genomic approaches to evaluate mechanisms of immune dysfunction in patients with R/R AML, with the goal of identifying biomarkers that predict for response to HIDAC/pembrolizumab, and novel pathways that lead to resistance to HiDAC/pembrolizumab in AML.
Results:

Patient Characteristics:

Between October 2016 and April 2019, 38 patients were enrolled and 37 treated with HiDAC followed by pembrolizumab (Table 1; Figure 1A). One patient received HiDAC but did not receive pembrolizumab due to infection, cellulitis, and grade 3 diarrhea and was thus replaced with another subject as per protocol criteria. Median age was 54 years with 41% of enrolled patients ≥60 years. The majority had either refractory (43%) or relapsed disease with CR duration <1 year (43%). All patients received intensive induction chemotherapy as front-line treatment with 76% receiving treatment on study as their first salvage therapy. Favorable, intermediate and adverse-risk disease by European Leukemia Net Classification was seen in 19%, 24% and 56% of patients, respectively. The most common genomic classification of patients on study were AML with NPM1 mutation (24%), AML with mutated chromatin, RNA-splicing genes or both (22%), and AML with MLL fusion genes (22%).

Toxicity:

Pembrolizumab was administered on day 14 in 32/37 (86%) patients. Three (8%) patients received pembrolizumab on day 15 and 2 (5%) patients received pembrolizumab on day 19 (Supplemental Table 1). The most common pembrolizumab-related toxicities were febrile neutropenia (62%), alanine aminotransferase (ALT) elevation (41%), hypocalcemia (30%), alkaline phosphatase elevation (30%), aspartate aminotransferase (AST) elevation (30%), hyperbilirubinemia (30%), lung infection (26%), and hypokalemia (24%). Most of these adverse events were grade 1-2 (Supplemental Table 2). Non-hematologic pembrolizumab-related grade ≥3 toxicities are shown in Table 2. Rare grade ≥3 iRAEs after pembrolizumab administration included maculopapular rash (n=2; 5%), aminotransferase elevation (n=2; 5%), lymphocytic infiltration on liver biopsy (n=1; 3%). Systemic steroids were administered to 5 (14%) patients for suspected grade ≥2 iRAEs (except for maculopapular rash) during induction phase.
(suspected grade 3 hepatitis: n=2, suspected grade 3 pneumonitis: n=1, grade 3 hemolytic anemia: n=1, grade 2 hyperbilirubinemia: n=1). Median time to administration of systemic steroids after pembrolizumab and total duration of steroids was 15 (range: 5-23) and 14 (range: 1-35) days, respectively. Steroids were rapidly tapered in 3/5 patients after diagnostic work-up revealed alternative etiologies or no evidence of an iRAE. Overall, iRAEs were self-limiting and fully resolved after administration of systemic steroids.

There was no treatment-related death on study. Overall 30-day and 60-day mortality was 0 and 3% (1 patient died on day 56 due to progressive disease), respectively. In those achieving a response, median time to full neutrophil (≥1x10^9/L) and platelet (≥100x10^9/L) recovery was 32 (range: 22-49) and 31 (range: 20-55) days, respectively.

Clinical Activity:
The ORR and CRc rates were 46% and 38%, respectively (Figure 1B). Of the 14 CRc patients, 13 achieved full hematologic recovery and 1 patient had incomplete platelet recovery (i.e, CRi). Seven (50%) of the 14 CRc patients had no evidence of measurable residual disease (MRD; Supplemental Table 3). CRc rates were encouraging in those receiving treatment as first salvage therapy (13/28 = 46%), <60 years (10/22 = 45%), and refractory AML (6/16 = 38%). Further, 9/25 (36%) patients with refractory or early relapse (CR1 duration ≤6 months) achieved CRc. Notably, 2/8 (25%) patients who were previously refractory to salvage chemotherapy with HiDAC prior to enrolling on study (n=1) or relapsed within 6 months of HiDAC consolidation (n=1) achieved CRc on this study, one of whom also had no evidence of MRD. In HiDAC-naïve patients, the CRc rate and median OS was 47% and 13.6 months, respectively (n=17; Supplemental Table 4).

Maintenance Phase:
Nine (24%) patients received pembrolizumab maintenance (median number of cycles = 3; range: 1-14) after achieving CR (n=8) or PR (n=1). Two patients subsequently received an alloSCT after 2 and 3 cycles of pembrolizumab maintenance, respectively. All 9 patients who received maintenance pembrolizumab developed relapse or progression (median time to relapse/progression = 5.8 months; range: 1.1-16.0 months). One patient achieved PR after HiDAC + pembrolizumab (17% blasts in BM) and remained with stable PR for 12 cycles of pembrolizumab maintenance. One patient achieved CR without MRD after HiDAC + pembrolizumab and developed flow cytometric MRD after cycle 12 before ultimately relapsing after cycle 14 of pembrolizumab maintenance.

No iRAEs or treatment-related grade ≥3 adverse events were observed during pembrolizumab maintenance (Supplemental Table 5). One patient developed grade 3 aseptic meningitis with a negative diagnostic work-up and symptoms improved with empiric antibiotics and supportive care without systemic steroids. One patient received empiric systemic steroids after developing acute onset grade 3 systolic heart failure, but after diagnostic work-up including catheterization, cardiac MRI and transmyocardial biopsy revealed no evidence of myocarditis, steroids were stopped. Both patients continued on pembrolizumab maintenance without difficulty. Thus, no patient discontinued maintenance due to toxicity.

Clinical Outcomes:

A swimmer plot of the 37 patients enrolled on study is illustrated in Figure 2A. 31/37 (84%) expired at data cut-off due to progressive AML (n=26) or infectious-related complications (n=5). Of the 6 patients alive at data cut-off, 3 received an alloSCT. Nine (24%) patients received an alloSCT after achieving CR (n=7), CRi (n=1), or morphologic leukemia-free state (MLFS: n=1). One patient who achieved CR without MRD relapsed and received an alloSCT after subsequent therapy. Six (67%) patients relapsed post-alloSCT (median time to relapse: 5.5 months; range: 1.3-23.6 months) including two who relapsed with extramedullary disease (Supplemental Table...
Two patients died of infectious complications 8.3 and 35.9 months post-alloSCT, respectively. There were no instances of sinusoidal obstruction syndrome or Grade ≥3 acute 
graft-versus-host in patients receiving pembrolizumab prior to alloSCT.

With a median follow-up of 15.1 months, the median OS was 11.1 months (95% CI: 6.3, 13.9 
months; Figure 2B). Median EFS and RFS was 6.7 months (95% CI: 4.9, 11.1 months), 5.8 
months (95% CI: 2.2, 10.4 months), respectively (Figure 2C-D). Additionally, median PFS was 
5.7 months (95% CI: 1.9, 10.4 months). Median OS was 14.4 months (95% CI: 13.2, N/A) 
among overall responders versus 5.0 months (95% CI: 4.0, 11.5 months) in those without a 
response to HiDAC + pembrolizumab. Further, median OS was 11.3 months (95% CI: 5.3, 20 
months) versus 5.0 months (95% CI: 2.9, N/A) in patients receiving no prior salvage therapy 
versus those receiving ≥1 prior salvage therapy, respectively. In patients with refractory disease 
or early relapse (CR1 duration ≤6 months), median OS was 13.2 months (95% CI: 5.2, 17.5 
months) compared with 7.0 months (95% CI: 4.0, N/A) in patients with late relapse (CR1 
duration >6 months).

Genomic Characteristics Associated with Response:

A plot of genomic signatures and mutations associated with response is shown in Figure 3. The 
CRc rate was 50% in those with AML with MLL fusion genes (4/8) and Inv(3)/t(3;3) (2/4), 
respectively (Supplemental Table 7). Notably, 2/5 (40%) patients with TP53 mutations achieved 
CR and 3/6 (50%) with ASXL1 mutations achieved an overall response (CR = 17%). In those 
with IDH1/2 mutations, ORR and CRc was 63% (5/8) and 25% (2/8), respectively. None of the 4 
patients with WT1 mutations had a response to treatment. Interestingly, all 3 patients without a 
detectable mutation achieved CR. ORR and CRc rate was 33% and 25%, respectively, among 
12 patients having genomic signature of secondary AML (20).

Immune Biomarker Correlates:
Previous work has shown that anti-PD-1 therapy may be most effective in patients with a diverse baseline T cell receptor (TCR) repertoire. We performed high-throughput antigen receptor sequencing of the TCR Vβ CDR3 region on sorted peripheral blood CD3+ T cells at diagnosis and analyzed them based on the response (CR, n=12; NR, n=11). Although greater than the pre-specified significance level of <0.05, TCR Vβ sequencing data revealed that patients who subsequently achieved CR had a trend towards higher TCR diversity at baseline compared with NR patients as assessed by Shannon Entropy (p=0.15; Figure 4A).

As the clonal repertoire determined by TCR sequencing of CD3+ T cells does not inform on functional differences between different T cell subpopulations, we next studied T cell dynamics by flow cytometry to probe cellular immune signatures that may correlate with response to HiDAC plus pembrolizumab treatment in paired BM and PB samples. Recognizing heterogeneity of dysfunctional T cells in AML that cannot be reliably assessed with a single phenotypic marker, we performed unsupervised clustering for cell subpopulation identification and UMAP for dimensionality reduction (Figure 4B-C). The advantage of this analysis lies in its integration of markers at a single-cell level, providing an improved understanding of their high-dimensional relationship. At baseline, we detected a significantly higher frequency of senescent T cells (CD45RA+KLRG1+CD57+) in the BM and PB and terminally differentiated effector T cells (TEMRA) cells in the PB in NRs as compared to those who achieved CR (Figure 4D; Supplemental Figure 1). Interestingly, patients who achieved CR had increased frequency of CD8+ T cells expressing CD28, PD-1, TIGIT, and lacking expression of Tim-3 and CD57 in the BM at baseline, a phenotype suggestive of progenitor exhausted T cells. Additional analysis using manual gating strategy (Supplemental Figure 2) confirmed that there is a statistically significant increase in pre-treatment CD8+CD45RA+CD27+/intCD28+PD1+TCF1+ T cells in patients who achieved CR compared to NRs (Figure 4E). TCF-1 is a transcription factor essential for the stem-like properties of intratumoral CD8+ T cells. The presence of CD8+ T cells
co-expressing TCF-1 and PD-1 appears to be critical for immunotherapy response.\(^{(24, 25)}\)

There were no significant differences observed in CD4\(^+\) T cell subpopulations at baseline and after treatment between CR and NR patients. However, there was a significant increase in T\(_{\text{reg}}\) in NR patients after treatment compared with baseline (Supplemental Figure 3). Sequencing data has been deposited in GEO under accession number GSE183415.

We next sought to examine transcriptional changes in enriched AML blasts using bulk RNA seq. Probing the Molecular Signature Database (MSigDB) and published gene sets revealed that at baseline, up-regulation of PI3K/AKT/mTOR signaling pathways in BM blasts was significantly associated with CR compared with NR patients by Gene Set Enrichment Analysis (GSEA) (Figure 5A). Notably, P53 pathway and inflammatory response pathways also showed up-regulation in CR compared to NR patients at baseline. On the other hand, MYC targets were significantly up-regulated in NR patients compared with CR. Genes with increased expression in CR included ARG1, which could regulate T cell metabolism by diminishing local concentrations of L-arginine (Figure 5B). Expression of multiple gene sets associated with Major Histocompatibility Complex (MHC-I/II) antigen presentation on pre-treatment leukemia blasts was significantly associated with CR (Supplemental Figure 4). There was no significant difference in PD-L1 or PD-L2 expression on blasts and non-blast fraction in CR versus NR patients, respectively (Supplemental Figure 5). Finally, there was no significant association with total mutational burden and CR versus NR (Supplemental Figure 6).

**Discussion:**

This study is the first to explore the use of ICB after cytotoxic salvage chemotherapy for patients with R/R AML. The study was designed as a phase II study with continuous monitoring for toxicity and early stopping rules for unacceptable toxicity given the uncertainty of administering ICB after salvage chemotherapy. Unacceptable toxicity, as defined in Methods (Safety Assessment), was not seen in any patient treated with pembrolizumab on this study. Despite
being administered during the time of nadir after HiDAC salvage, pembrolizumab administration was associated with rare iRAEs, no severe toxicity or delay in hematologic recovery, and an impressive 30-day mortality rate of 0%. In fact, only 5 (14%) patients experienced a grade ≥3 iRAE which all resolved with steroids and/or supportive care. In contrast to data in solid malignancies where pneumonitis occurs in up to 10% of patients treated with PD-1 inhibitors(26), there were no incidences of pneumonitis after pembrolizumab administration on this study, both during induction and maintenance. Rigorous diagnostic work-up of suspected iRAEs led to alternative etiologies in several patients reinforcing the importance of ruling out more likely alternative causes in AML patients treated with ICB.

The overall CRc rate of HiDAC + pembrolizumab was 38%, meeting the primary endpoint of this study, and median OS was 11.1 months thus substantiating clinical activity of this regimen and comparing favorably to other salvage regimens. This study was designed based on a historical control CR rate of 20% with HiDAC alone.(4, 5) Reported clinical studies of salvage chemotherapy regimens in R/R AML are fraught with heterogeneity of patient populations, disease settings, chemotherapy regimens used, and outcomes. While response rates as high as 58%(27, 28) have been reported in single-arm phase II studies, randomized phase II-III studies with multi-agent cytotoxic chemotherapy have consistently shown CR rates of ≤35% and median OS <8 months in patients with R/R AML.(1, 4-6) Further, in HiDAC-naïve patients, CRc and median OS was 47% and 13.6 months, respectively, which compare favorably to HiDAC-naïve treated patients on 3 randomized phase 3 studies (CRc = 12-32%; median OS = 5-8 months; Supplemental Table 4).(5, 29, 30) We chose to use HiDAC as a single agent in this study in order to attenuate lymphocyte depletion and T cell suppression that can be seen after purine analogs such as fludarabine, clofarabine and cladribine(31) which are commonly used in salvage chemotherapy regimens for AML. Whether ICB would be clinically active after more
conventional multi-agent cytotoxic salvage chemotherapy regimens is unclear and worthy of further investigation.

R/R AML encompasses a heterogeneous group of patients with variably poor clinical outcomes. Primary refractory AML and early relapse (typically defined as CR duration ≤6 months) represent a particularly dismal subgroup of R/R AML with CR rates and median OS of approximately 14% and 3-4 months, respectively. Only 5 (14%) patients on this study had a CR1 duration >1 year while 68% of patients had either refractory AML or early relapse (CR1 duration ≤6 months). The clinical outcomes of this highest-risk subgroup of patients with refractory/early relapse AML was particularly encouraging with CRc of 36% and median OS of 13.2 months. This patient population is inherently chemo-resistant and represents one of the highest unmet needs in AML. Higher levels of expression of IFN-γ-related genes and an immune infiltrative tumor microenvironment with increased expression of immune checkpoints are hallmarks of refractory AML and may define a subset of patients who respond best to immunotherapy. Recent data suggests that patients with primary refractory/early relapse AML may have improved responses to immune based therapies, such as flotetuzumab, compared to late relapse patients, due to higher BM immune infiltration, inflammatory chemokine, expression of IFN-γ-related genes, and tumor inflammation signature scores. Thus, immune intervention with pembrolizumab may have reactivated T cell responses in this refractory patient population and contributed to anti-leukemia activity of this regimen.

Encouraging clinical outcomes were also seen in patients receiving HiDAC plus pembrolizumab as their first salvage therapy with ORR and median OS rates of 54% and 11.3 months, respectively. Daver et al. also reported improved outcomes in R/R AML patients receiving azacitidine plus nivolumab as their first salvage therapy when compared to historical controls receiving azacitidine alone (median OS = 10.6 vs. 5.3 months, respectively). These data,
therefore, suggest that PD-1 inhibitors should be incorporated into combination therapies during earlier lines of therapy in R/R AML.

Higher CD3$^+$ T cells in PB/BM have been associated with responses to azacitidine and nivolumab.(37) In our study, we did not observe correlation between baseline BM and PB CD3$^+$ T cells and response possibly due to small sample size. However, we observed higher frequency of highly differentiated and senescent T cells in non-responders to therapy. We previously reported that this T cell subpopulation is less proliferative and ineffective in killing leukemia cells.(11) Further, TCR repertoires of patients who achieved CR were more diverse than those who did not respond to therapy, suggesting that pre-treatment TCR repertoire diversity may imply a greater likelihood of response. Similar results were seen with ipilimumab in melanoma and PD-1 blockade in classical Hodgkin lymphoma.(21, 38) Further probing of immune signatures revealed enrichment of progenitor exhausted TCF1$^+$ CD8$^+$ T cell subpopulation in patients who achieved CR. This population had been described to give rise to effector cells after PD-1 inhibition. Higher percentages of progenitor exhausted CD8$^+$ T cells in the tumor microenvironment pre-treatment have also been reported in patients with melanoma whom experienced durable responses to ICB.(25, 39) This finding suggests that T cell differentiation state rather than the sole number of T cells may be predictive of response to PD-1 inhibition in AML.

Interestingly, CR in this study was associated with increased expression of the PI3K/AKT/mTOR pathway in BM blasts. Previous evaluations have predominantly focused on the role of mutations in PI3K in the immune response in cancer with little data on the effects of increased expression of this pathway. However, mutations of PTEN lead to activation of PI3K and in murine lung cancer models led to upregulation of PD-L1.(40) Treatment with combined ICB and an mTOR inhibitor led to increased CD8$^+$ T cells, decreased T$_{reg}$ and improved tumor control.(41) Notably, none of the 4 patients with WTI mutations achieved a response. Becker et
al(42) revealed that WT1 mutations were associated with a down-regulation in genes involving PI3K pathway purporting a potential mechanism of resistance to PD-1 inhibition. Future studies assessing the combination of ICB and mTOR inhibition are warranted in AML.

In terms of genomic predictors of response, small numbers of patients on this study precluded a robust analysis but a few observations are noteworthy. We observed ORR of 50% in patients with ASXL1 mutations in our study. ASXL1 mutations were associated with improved ORR and OS in a phase II study of azacitidine plus nivolumab in R/R AML.(37) Two of four patients with inv(3)/t(3;3) cytogenetics, which has been shown to be the most adverse-risk abnormality in AML,(22) achieved CRc on study and subsequently received an alloSCT. Patients with inv(3)/t(3;3) frequently harbor mutations in RAS/receptor tyrosine kinase pathways and notably have higher mutational burden than other AML subtypes.(43) Lastly, two of five patients enrolled with TP53 mutations achieved CRc. TP53-mutated AML have particularly poor outcomes with conventional chemotherapy agents yet novel immunotherapy agents have shown promising clinical activity in this patient subset. (44, 45) Recent data suggests that TP53 mutations as well as MDS-like subtypes are associated with high cytolytic scores and PD-L1 expression (46) suggesting that future studies of novel immune-based combinations deserve exploration in TP53 mutant AML patients.

There were several limitations of this study that will require further investigation in future study designs. First, the majority of patients (76%) who enrolled on this study received only a single dose of pembrolizumab. Incorporation of serial doses of PD-1 inhibitors, with or without other ICB agents or immune therapies, and in those without an initial response to therapy may be required given the potential for delayed onset of clinical activity and to overcome an immunosuppressive BM microenvironment. Ravandi et al (47) reported encouraging clinical outcomes and rare iRAE’s despite serial doses of ICB in a phase II study of nivolumab after induction chemotherapy in newly diagnosed AML and high-risk myelodysplastic syndrome.
Second, a primary endpoint of CR after 1 cycle of therapy may underestimate the clinical activity of ICB in AML and attentive study designs will be necessary to allow adequate time for immune modulation. As a case in point, prolonged response has been observed in patients who achieved PR and MLFS on this study and was comparable to those who achieved CRc (median RFS and PFS 5.8 vs. 5.7 months, respectively). In fact, recent data suggests that ICB may sensitize patients with Hodgkin Lymphoma to subsequent therapies after progression; thus, the impact of ICB may last beyond initial disease evaluations and response.(48) Third, uniform MRD monitoring was not performed on this study, in part, due to lack of standardized assessment of MRD in AML. Nonetheless, achievement of CR without MRD is associated with improved clinical outcomes(49) and it would be of interest to examine the effect of pembrolizumab and other ICB therapies on the depth of response over time, as is being done in two randomized clinical trials (NCT04214249, NCT04284787). Lastly, correlative immunologic studies, while informative in identifying the relevance of distinct subpopulation of progenitor exhausted T cells in relation to response, are limited by small number of available patients for analysis and relatively few sampling time points. Prospective assessment of NK cell function was not performed in this study and is warranted for future exploration.

In conclusion, the addition of pembrolizumab to HiDAC salvage chemotherapy in R/R AML led to an acceptable safety profile, met the primary endpoint of achieving clinical activity with CRc rate of 38%, and led to encouraging clinical outcomes with median OS of 11.1 months. Patients with refractory/early relapse AML and those receiving HiDAC plus pembrolizumab as their first salvage regimen appeared to have the greatest benefit. Based on these findings, a randomized phase II study of salvage chemotherapy plus pembrolizumab versus salvage chemotherapy alone in refractory/early relapse AML is warranted, including prospective examination of the progenitor exhausted TCF1+ CD8+ BM T cell subpopulation as a potential predictor of response.
Materials and Methods:

Study Design and Population:

This study was an open-label, single-arm, phase II study of HiDAC followed by pembrolizumab in patients with R/R AML conducted at two institutions: University of North Carolina, Lineberger Comprehensive Cancer and Johns Hopkins Hospital, Sidney Kimmel Comprehensive Cancer Center (clinicaltrials.gov identifier NCT02768792). Eligible patients included those 18-70 years with pathologically confirmed refractory or relapsed AML, defined by ≥5% myeloblasts in bone marrow (BM) aspirate and/or biopsy. Patients must have received first-line treatment with ≥1 cycle of intensive induction chemotherapy or ≥4 cycles of hypomethylating agents prior to enrollment. Patients with acute promyelocytic leukemia and those who have received an alloSCT prior to enrollment were excluded. Detailed eligibility criteria are outlined in Supplemental Methods. All laboratory criteria for eligibility must have been met prior to enrollment and before pembrolizumab administration (see Treatment Plan below). The study was conducted in accordance with Declaration of Helsinki after approval by ethics committee of each participating center. Written informed consent was obtained on all subjects prior to participating.

Treatment Plan:

Induction Phase:

All patients received HiDAC at the following dose levels: 18-59 years: HiDAC 2 gm/m² IV every 12 hours days 1-5; 60-70 years: HiDAC 1.5 gm/m² IV every 12 hours days 1-5 (Figure 1). HiDAC was permitted to be dose-reduced and/or discontinued due to organ dysfunction (i.e. renal or hepatic abnormalities) per institutional standards. Patients must have received >50% planned doses of HiDAC to remain eligible for pembrolizumab administration. Pembrolizumab
200 mg IV was administered on day 14 of treatment. Prior to initiation of pembrolizumab, patients were required to meet laboratory eligibility (see Supplemental Methods). If patients did not meet laboratory eligibility criteria and/or had uncontrolled intercurrent illness deemed by the investigator to be unsafe to administer pembrolizumab, pembrolizumab administration was permitted to be delayed up until day 21. Subjects ineligible to receive pembrolizumab by day 21 of therapy were removed from study protocol and replaced by another subject. All patients were hospitalized for treatment and discharged once early hematologic recovery was achieved. 

**Maintenance Phase:**

Patients who achieved a partial remission (PR), CR, or CR with incomplete recovery (CRi: see Assessment of Response) were eligible to receive maintenance phase pembrolizumab 200 mg IV every 3 weeks for up to 2 years until disease relapse, subsequent therapy (including alloSCT), or death. Prior to initiation of maintenance phase, all patients were required to meet laboratory-based eligibility criteria (see Supplemental Methods) and all treatment-related toxicities must have resolved to ≤ Grade 1. Subjects with unacceptable toxicity (see Safety Assessment) during induction phase were not eligible to receive maintenance phase. Maintenance phase was permitted 10-60 days after full hematologic recovery from induction. Patients could undergo alloSCT after salvage treatment or during maintenance phase but stem cell infusion day 0 was required to be ≥21 days after last dose of pembrolizumab.

**Safety Assessment:**

All patients who received pembrolizumab were evaluable for safety. Toxicity was assessed by NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. Continuous monitoring for toxicity was performed throughout the study after pembrolizumab administration. Sequential boundaries were used to monitor unacceptable toxicity rates secondary to pembrolizumab. An unacceptable toxicity was defined as any drug-related grade 3 non-hematologic toxicity (exceptions include infusion reactions, rash, fever, infection, nausea,
fatigue and anorexia) persisting for >7 days despite supportive care, or any drug-related grade 4-5 non-hematologic toxicity (excluding infection). The accrual was halted if excessive numbers of unacceptable toxicities was equal to or exceeded boundary \( b_n \) out of \( n \) patients with full follow-up (Supplemental Table 8). A Pocock-type stopping boundary was used and yields the probability of crossing the boundary at most 0.05 when the rate of unacceptable toxicity is equal to the acceptable rate of 0.2. The stopping boundary guided enrollment as well as suspension of accrual (i.e., when to stop the trial if necessary). Initially, 3 patients were enrolled and accrual was then held until at least one of the first 3 patients completed follow-up (i.e. full hematologic recovery or no response to treatment) and was confirmed not to have unacceptable toxicity.

Dose modifications and management guidelines of immune-related adverse events (iRAEs) secondary to pembrolizumab are outlined in Supplemental Table 9. Drug-related grade \( \geq 2 \) iRAEs (except for rash, infusion reactions and thyroid dysfunction) were treated with systemic corticosteroids until resolution to grade \( \leq 1 \). When possible, pathologic confirmation of iRAEs were recommended prior to initiation of systemic corticosteroids.

**Assessment of Response:**

A BM aspirate and biopsy were performed at the time of full hematologic recovery (i.e. absolute neutrophil count: \( \text{ANC} \geq 1 \times 10^9/L \) and platelets \( \geq 100 \times 10^9/L \)) or by day 45 of induction phase. Response criteria were consistent with standardized guidelines by European Leukemia Net.(50) MRD testing was done by institutional standards at the time of each response assessment. BM aspirate and biopsy were performed after every 4 cycles (i.e. every 3 months) of pembrolizumab for the first year, after every 6 cycles (i.e. every 4.5 months) for second year of maintenance phase and at any point of clinical suspicion of relapse.

**Next-Generation Sequencing (NGS):**
DNA was extracted from either the sorted blast populations or unsorted bulk cells, prepared in each case from BM aspirate samples collected prior to treatment on study (n=31) or during prior lines of therapy (n=6). NGS was then performed using a 34-gene, customized hybridization capture assay (Custom Myeloid Solution, SOPHiA Genetics, Lausanne, Switzerland). Further methodology of the NGS panel and a full list of targeted genes and exons is included in the Supplemental Methods and Supplemental Table 10, respectively.

Immune Biomarker Correlates:

Mononuclear cells were isolated from PB and BM aspirates by Ficoll-Hypaque gradient centrifugation and cryopreserved. AML blasts were further enriched for RNA seq analysis (Supplemental Methods). DNA and RNA were extracted from selected AML blast cells with AllPrep DNA/RNA Mini kit (Qiagen Cat. No. 80204). Samples of total RNA were extracted from AML blasts from BM aspirates by Qiagen RNeasy. Illumina TruSeq RNA Access sequencing libraries were created to convert total RNA into template molecules followed by sequence-specific capture of coding RNA. Sequencing was performed on an Illumina HiSeq 4000 platform using the Illumina HiSeq SBS 150 cycles with paired end 2 x 75 base read pairs.

Samples of total RNA extracted from CD8⁺ bead selected lymphocytes from peripheral blood mononuclear cells (PBMCs: Qiagen RNeasy Plus mini Cat. No 79134 if >500,000 cells, or RNeasy Plus micro if <500,000 cells Cat No. 74034) were used to prepare mRNA stranded sequencing libraries (Illumina Tru-Seq Stranded Library Prep Kit Cat. No 20020594). Enrichment procedures of CD8⁺ lymphocytes for adaptive immune receptor repertoire analysis and mRNA stranded sequencing libraries are outlined in Supplemental Methods.

Flow Cytometry:

BM mononuclear cells (BMMCs) and PBMCs were serially collected from AML patients (BM, n = 20; PB, n = 21) at baseline and at the time of response assessment (see Assessment of
Response) after HiDAC plus pembrolizumab. Flow cytometry was performed on a BD-Fortessa (Becton Dickinson) provided with BD FACSDiva software (Becton Dickinson) version 8.0.1. Antibodies used for analysis are listed in Supplemental Table 11. Flow cytometry data were biexponentially transformed, compensated using single stained controls and preprocessed (aggregates and dead cell removal) in FlowJo V10 (TreeStar). The percentage of CD4\(^+\) and CD8\(^+\) T cells and other tertiary markers on each T cell sub-population were analyzed on GraphPad Prizm software Version 7. Pre-gated CD8\(^+\) T cells were then exported in R (version 4.0.2) for further analyses performed with a customized pipeline based on Nowicka M et al.\(^{(51)}\) workflow. In particular, CD8\(^+\) T cells clusters were obtained using the FlowSOM algorithm and then visualized using the implementation of Uniform Manifold Approximation and Projection (UMAP) available in CATALYST R package. The different frequencies of the T cell subpopulations in CR and non-responders (NR) at the two timepoints (baseline and after treatment) were identified using the differential abundance analysis provided by the diffcyt R package \(^{(52)}\). Further details are listed in Supplementary Methods.

**Statistical Assessment:**

The primary objective of this single-arm open-label phase II study was to estimate the composite CR (CRc: CR + CRi) rate of HiDAC followed by pembrolizumab in R/R AML. The study design was a Simon’s like two-stage design with relaxed stopping for futility. Relaxed stopping refers to inclusion of PR in the first stage as some of these PRs may convert to a CR during maintenance phase. The null hypothesis that the true CRc rate for HiDAC followed by pembrolizumab is 20% was tested against a one-sided alternative hypothesis. In the first stage, 19 patients were enrolled. If the number of patients who achieved a CRc plus the number of patients with PR was equal to \(<4\) in these 19 patients, the study would be stopped for futility. Otherwise, 18 additional patients were enrolled for a total of 37 patients. The null hypothesis will be rejected if \(\geq12\) CRc's are observed in 37 patients. Assuming that the PR rate has a uniform
distribution, this design yields a type 1 error rate of at most 5% and power of at least 84% when the true CR rate for HiDAC followed by pembrolizumab is 40%.

Secondary endpoints of this study included rates of unacceptable toxicity as defined in Safety Assessment, toxicity of HiDAC + pembrolizumab induction phase and pembrolizumab maintenance, objective overall response rates (ORR: CR + CRi + PR + MLFS), OS defined as day 1 of treatment until date of last known follow-up, relapse-free survival (RFS) defined as time from CRc until relapse or death, progression-free survival (PFS) defined as time from ORR until relapse, progression, or death, and event-free survival (EFS) defined as day 1 of treatment until no response, relapse or death. Survival measurements were summarized by Kaplan-Meier methodology. Database lock was done on May 1, 2020.

Acknowledgements:

This study was supported in part by a research grant from Investigator-Initiated Studies Program of Merck, Sharp & Dohme Corp. The opinions expressed in this paper are those of the authors and do not necessarily reflect those of Merck Sharp & Dohme Corp.

This data was presented, in part, at the annual American Society of Hematology (ASH) Meeting in 2017, 2018, 2019, and 2020.

The authors would like to thank the research staff and co-investigators at the University of North Carolina and Johns Hopkins for their invaluable contribution to this study. We would also like to thank the patients and their families who have participated in this study.
References:


Table 1: Patient Characteristics

<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>N=37</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age- Median (Range)</td>
<td>54 (24-70)</td>
</tr>
<tr>
<td>&gt;55 years</td>
<td>17 (46%)</td>
</tr>
<tr>
<td>&gt;60 years</td>
<td>15 (41%)</td>
</tr>
<tr>
<td>Male</td>
<td>20 (54%)</td>
</tr>
<tr>
<td>Female</td>
<td>17 (46%)</td>
</tr>
<tr>
<td>BM Blast % prior to treatment- Median (Range)</td>
<td>28% (6-94%)</td>
</tr>
<tr>
<td>Refractory AML¹</td>
<td>16 (43%)</td>
</tr>
<tr>
<td>Relapsed AML</td>
<td>21 (57%)</td>
</tr>
<tr>
<td>CR duration ≤6 months</td>
<td>9/21 (43%)</td>
</tr>
<tr>
<td>CR duration ≤1 year</td>
<td>16/21 (76%)</td>
</tr>
<tr>
<td>Salvage Therapy 1</td>
<td>28 (76%)</td>
</tr>
<tr>
<td>Salvage Therapy 2</td>
<td>8 (22%)</td>
</tr>
<tr>
<td>Salvage Therapy 3</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Secondary AML</td>
<td>13 (35%)</td>
</tr>
<tr>
<td>ELN Risk</td>
<td>7 (19%)</td>
</tr>
<tr>
<td>Favorable</td>
<td>9 (24%)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>21 (56%)</td>
</tr>
<tr>
<td>Adverse</td>
<td>4 (11%)</td>
</tr>
<tr>
<td>Genomic Classification²</td>
<td>9 (24%)</td>
</tr>
<tr>
<td>AML with NPM1 mutation</td>
<td>8 (22%)</td>
</tr>
<tr>
<td>AML with mutated chromatin, RNA splicing genes or both</td>
<td>8 (22%)</td>
</tr>
<tr>
<td>AML with MLL fusion genes</td>
<td>6 (16%)</td>
</tr>
<tr>
<td>AML with TP53 mutations, chromosomal aneuploidy, or both</td>
<td>4 (11%)</td>
</tr>
<tr>
<td>AML with Inv(3); GATA2, MECOM</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>AML with Inv(16); CBFB-MYH11</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>AML with t(6;9); DEK-NUP214</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Most Common Mutations</td>
<td>9 (24%)</td>
</tr>
<tr>
<td>NPM1</td>
<td>8 (22%)</td>
</tr>
<tr>
<td>DNMT3A</td>
<td>6 (16%)</td>
</tr>
<tr>
<td>ASXL1</td>
<td>5 (14%)</td>
</tr>
<tr>
<td>IDH2</td>
<td>4 (11%)</td>
</tr>
<tr>
<td>TP53</td>
<td>4 (11%)</td>
</tr>
<tr>
<td>CEBPA</td>
<td>4 (11%)</td>
</tr>
<tr>
<td>NRAS</td>
<td>4 (11%)</td>
</tr>
<tr>
<td>WT1</td>
<td>4 (11%)</td>
</tr>
<tr>
<td>Site</td>
<td>30 (81%)</td>
</tr>
<tr>
<td>University of North Carolina</td>
<td>7 (19%)</td>
</tr>
<tr>
<td>Johns Hopkins</td>
<td>7 (19%)</td>
</tr>
</tbody>
</table>

1- Refractory AML- defined as no response to 1 or 2 cycles of induction chemotherapy (>28 days after induction chemotherapy) or no response to salvage treatment after subsequent relapse
2- Genomic Classification determined by Papaemmanuil et al.(19)
Table 2: Treatment-Related Grade ≥3 Non-Hematologic Adverse Events After Pembrolizumab

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Number (Total %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrolyte Abnormalities</td>
<td></td>
</tr>
<tr>
<td>Hypokalemia</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Hepatic</td>
<td></td>
</tr>
<tr>
<td>Alanine Aminotransferase Increase</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Aspartate Aminotransferase Increase</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Alkaline Phosphatase Increase</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Lymphocytic infiltration of liver</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Infections</td>
<td></td>
</tr>
<tr>
<td>Catheter-related infection</td>
<td>3 (8%)</td>
</tr>
<tr>
<td>Clostridium difficile colitis</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Febrile neutropenia</td>
<td>23 (62%)</td>
</tr>
<tr>
<td>Hepatic infection</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Lung infection</td>
<td>10 (26%)</td>
</tr>
<tr>
<td>Typhlitis</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Pulmonary</td>
<td></td>
</tr>
<tr>
<td>Pulmonary edema</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Skin</td>
<td></td>
</tr>
<tr>
<td>Maculopapular rash</td>
<td>2 (5%)</td>
</tr>
</tbody>
</table>

The proportion of grade ≥3 non-hematologic adverse events related to pembrolizumab are shown in this Table.
Figure 1

A) Treatment Schema

*1 patient received HiDAC but did not receive pembrolizumab due to ongoing grade 3 diarrhea and was thus replaced on this study.

B) Overall response rates among different patient subsets.
Figure 2: Clinical Outcomes

A) Swimmer plot of best treatment response and survival for all 37 patients. The swim lanes (rows) represent patients in the study and their survival until date of last follow-up or death.

End of response = relapse (after CR/CRi) or progression (after PR).

B) Kaplan-meier estimates of Overall survival (OS) measured from day 1 of treatment until death or date of last follow up.

C) Kaplan-meier estimates of Event-free survival (EFS) measured from day 1 of treatment until no response, relapse or death.

D) Kaplan-meier estimates of Relapse-free survival (RFS) measured from date of CR/CRi until relapse, death or date of last follow up.
Figure 3: Genomic Signatures and Response Matrix

Gene matrix representing mutations identified by NGS prior to treatment. Each individual patient is listed as a column on the X axis. Mutations identified as present prior to treatment are colored in black. Thirty-one patients had NGS performed as baseline prior to treatment on study (dark grey); whereas, 6 patients (light grey) had NGS performed during prior lines of therapy. Genomic classification was determined based on Papaemmanuil et al.(19). Genomic signature of secondary AML was determined based on Lindsley et al(20) (Beige color denotes patients with secondary AML based on genomic signature). Mutations were listed in order of prevalence on the y axis. The percentage listed in the last column represents prevalence of the gene mutation in the overall cohort.
Figure 4: Immune Biomarkers Associated with Response

A) Shannon Entropy of CR (n=12) and NR (n=11) pre-treatment PB TCR samples. Uncorrected p value = 0.15 comparing CR versus NR patients. TRB = TCR Vβ

B) Heatmap showing the 0-1 scaled MFI values of 12 markers over the eight CD8+ BM subsets from all samples (NR: n=11; CR: n=8). The median marker expression identifies the markers that characterize each cell subset. Each CD8+ subpopulation is colored according to the cluster identified using the FlowSOM algorithm.

CD8 activated effector: DNAM1+CD28*KLRG1*CD69*CD56+
CD8 partially senescent: CD28*CD27*KRLG1*CD57+
CD8 progenitor exhausted: CD28*PD1*TIGIT*
CD8 TEMRA CD57+: CD45RA*KLRG1*CD57+
CD8 senescent: CD45RA*KLRG1*CD57+
CD8 DNAM1*CD28+: DNAM1*CD28+
CD8 DNAM1*PD1+: DNAM1*PD1+
CD8 naive: CCR7*CD45RA*CD27*CD28+

C) UMAP visualization overlaid with contour plots (kernel density estimation) of the eight CD8+ BM subpopulations in non-responders (NR, n = 11) and complete responders (CR, n = 8), at baseline (base_CR, base_NR) or after therapy (post_CR, post_NR). Each CD8+ subpopulation is colored according to the cluster identified using the FlowSOM algorithm.

D) Boxplots showing the relative abundance of BM CD8+ subpopulations in NR and CR patients at baseline and post-treatment. Horizontal bars indicate median values. Asterisks indicate adjusted p-values (*padj< 0.05).

E) Frequency of BM CD8+CD45RA-CD27/intCD28*PD1*TCF1+ T cells in patients who achieved CR compared to NRs at baseline and at response assessment (*p< 0.05).
Figure 5:

A) Gene Set Enrichment Analysis of pre-treatment BM blast samples using the Hallmark gene sets from the Molecular Signatures Database (http://www.gsea-msigdb.org/gsea/msigdb/collections.jsp).(53)

B) Heatmap displaying the differential expression of genes from pre-treatment BM blast RNA samples comparing CR versus NR patients. FDR p≤0.20 shown.
A

Age <60 years:
HiDAC 2 gm/m² IV
Q12hours D1-5
• n=22

Age 60-70 years:
HiDAC 1.5 gm/m² IV
Q12hours D1-5
• n=16

Pembrolizumab 200 mg IV day 14
• n=37*

Pembrolizumab
200 mg IV
Q3weeks
• n=5

n=38 patients

Screening
HiDAC salvage
Enrollment on study
Maintenance Phase

B

Response Among Subgroups

Response Rate

Overall 38 46 45 26 38 38 31 42
Salvage 1 11
Salvage 2+ <60 years >=60 years Refractory Relapse Secondary AML de novo AML

Figure 1
Figure 2
Figure 4
Phase II Trial of Pembrolizumab after High Dose Cytarabine in Relapsed/Refractory Acute Myeloid Leukemia

Joshua F Zeidner, Benjamin G. Vincent, Anastasia Ivanova, et al.

Blood Cancer Discov  Published OnlineFirst September 10, 2021.

Updated version  Access the most recent version of this article at:
doi: 10.1158/2643-3230.BCD-21-0070

Supplementary Material  Access the most recent supplemental material at:
http://bloodcancerdiscov.aacrjournals.org/content/suppl/2021/08/28/2643-3230.BCD-21-0070.DC1

Author Manuscript  Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, use this link http://bloodcancerdiscov.aacrjournals.org/content/early/2021/09/07/2643-3230.BCD-21-0070. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.