A phase II study of post-remission therapy with pembrolizumab in older patients with acute myeloid leukemia

Leukemic relapse remains a major cause of mortality among older patients with acute myeloid leukemia (AML) who are not candidates for allogeneic hematopoietic stem cell transplantation (HSCT). In this population, hypomethylating agents, and in particular orally administered azacitidine, may be used as maintenance therapy to prolong remissions, with the latter receiving Food and Drug Administration (FDA) approval after the demonstration of an improvement in median relapse-free survival of over 5 months compared to that in patients given a placebo.[1] Nonetheless, most patients ultimately relapse and therefore alternatives for maintaining remission are needed in this population.[1,2]

A proven approach for re-invigorating antitumor activity by T cells has been to block the interactions of programmed cell death protein 1 (PD-1), expressed by T cells, and programmed death-ligand 1 (PD-L1), which is commonly expressed and/or upregulated by cancer cells to suppress T-cell activation.[3] In preclinical models of AML, PD-1 was upregulated in the presence of murine leukemia cells and antibody-mediated blockade of PD-L1 suppressed *in vivo* leukemia cell proliferation and improved survival in AML-bearing mice.[4] Expression of inhibitory markers was shown to be greater among AML patients with multiple relapse events compared to those with newly diagnosed disease and increased expression of these immune checkpoint proteins was associated with immune exhaustion which could contribute to leukemia relapse.[5,6] Pembrolizumab, a humanized mouse-derived anti-PD-1 antibody that promotes T-cell mediated tumor-cell apoptosis by disrupting PD-1/PD-L1 interactions, is approved for use in patients with solid cancers and hematologic malignancies.[7] In AML, pembrolizumab has been investigated with decitabine, or as maintenance therapy for those achieving complete remission (CR) after high-dose cytarabine for relapsed/refractory disease in early phase trials with favorable safety profiles.[8,9] We hypothesized that immunomodulation with the use of pembrolizumab after remission could promote an antileukemia immune response and prevent or delay leukemia relapse. Here, we conducted a single-arm phase II trial using pembrolizumab administered every 3 weeks for up to 2 years or until disease relapse as post-remission therapy to prevent leukemia relapse in older AML patients. We also explored the effect of pembrolizumab on immune cell function in these patients. Eligible patients had to be at least 60 years of age with newly diagnosed AML and to have achieved CR or CR with incomplete hematologic recovery (CRi) following induction therapy, as documented by bone marrow biopsy. Patients with isolated t(8;21), inv(16) or t(16;16)(p13.1;q22) or mutated *NPM1* with wild-type *FLT3* and no additional mutations were excluded. All patients were required to have completed induction chemotherapy, with or without consolidation therapy, within 3 months of enrollment. Additionally, patients were required to have an Eastern Cooperative Oncology Group (ECOG) performance-status score of 0 or 1, an absolute neutrophil count ≥1.0×10^9/L and platelet count ≥50×10^9/L at the time of enrollment. The study was approved by the University of Pittsburgh Institutional Review Board and all patients signed written informed consent. The trial was registered with ClinicalTrials.gov (NCT02708641).

Twelve patients with AML were enrolled between 2015 and 2019, before FDA approval of oral azacitidine maintenance therapy.[1] Patient and disease characteristics are included in Table 1. The mean age was 68.5 years (range, 65.5-73.5 years) and most patients had *de novo* AML (66%). Two patients were classified as having favorable-risk disease by European LeukemiaNet 2017 criteria, both with mutated *NPM1* and wild-type *FLT3*, with mutated *DNMT3A*. Eight patients were in CR and four patients in CRi at enrollment. Measurable residual disease (MRD) by flow cytometry (sensitive to 1 in 10^4 cells) was evaluable from bone-marrow aspirates in ten patients prior to starting pembrolizumab and all were MRD negative. Longitudinal MRD assessments were not performed during the study. All patients were induced with intensive regimens, with 75% receiving the standard idarubicin and cytarabine ("7+3") regimen. Nine patients (75%) received at least one dose of consolidative high-dose cytarabine. At the discretion of the treating physician, three patients (25%) did not receive consolidation chemotherapy before enrollment in the study as they were deemed to be poor candidates for additional cytotoxic chemotherapy.

The median duration of treatment with pembrolizumab was 12 cycles (range, 2-35), with a median time to relapse of 12.3 months (Figure 1A) and a median overall survival of 43.1 months (Figure 1B). Two patients completed 2 years of maintenance pembrolizumab and seven discontinued therapy due to leukemia relapse. Five patients (41%) developed immune-related adverse events requiring interventions, of whom four discontinued pembrolizumab. At the time of analysis, no patients were continuing maintenance pembrolizumab. Among the eight patients who relapsed, one underwent re-induction and allogeneic HSCT, achieving a durable second CR, whereas the remaining relapsed patients died from their disease (Figure 1C). In subgroup analyses, the median time to relapse was significantly longer among patients with ECOG 0 performance status at the time of

enrollment than in those with ECOG 1: 18.7 months *vs*. 3.0 months, respectively (*P*=0.00018). The median time to relapse of those who received consolidation therapy was also significantly improved compared to those who did not, 18.8 months *vs*. 4.2 months, respectively (*P*=0.019) (*Online Supplementary Figure S1A*). No relapses were observed among two patients with favorable-risk disease, as defined by European LeukemiaNet criteria, whereas the median time to relapse of intermediate-risk patients was 14.0 months and that of adverse-risk patients, 4.2 months (*P*=0.034). The median overall survival was similarly correlated with performance status (not reached [NR] for ECOG 0 patients *vs*. 6.7 months for ECOG 1 patients; *P*=0.0032), receipt of consolidation therapy (NR for those who received consolidation *vs*. 7.0 months for those who did not receive consolidation; *P*=0.007), and leukemia risk (NR, NR, and 7.0 months, for those with favorable, intermediate and adverse risk, respectively; *P*=0.011) (*Online Supplementary Figure S1B*).

Among the immune-related adverse events requiring intervention, one patient developed grade 2 thyroiditis after cycle 3 that required treatment with thyroid hormone replacement; one patient developed grade 2 pneumonitis with viral respiratory infection after cycle 13 that improved with antiviral therapy and discontinuation of pembrolizumab; one patient developed grade 4 immune thrombocytopenia after cycle 19 that responded to steroids and discontinuation of pembrolizumab; and one patient developed colitis after cycle 13 with grade 3 diarrhea that responded to steroids and discontinuation of pembrolizumab. Finally, one patient developed grade 3 aspartate aminotransferase elevation and constitutional symptoms after cycle 12 which resolved with discontinuation of the treatment. *Online Supplementary Table S1* summarizes the adverse events over the course of this trial.

Prior to administration of pembrolizumab, the frequencies of T cells in peripheral blood ($CD4^+$, $CD8^+$ and $CD25^+FOXP3^+$ regulatory T cells) were similar between patients who ultimately relapsed and those who did not (Figure 2A). After the first cycle of pembrolizumab there was a significant (*P*<0.02) decrease in the percentage of peripheral $FOXP3^+$ regulatory T cells among $CD4^+CD25^+$ cells in all patients which was transient and did not persist in later cycles (Figure 2B). Furthermore, this decrease did not reach statistical significance when $FOXP3^+$ regulatory T cells were analyzed in patients who ultimately relapsed and those who did not were analyzed in separate cohorts. Additionally, no significant changes in the frequency of $CD4^+$ or $CD8^+$ T cells were observed following therapy (Figure 2B) and pembrolizumab did not affect $CD4^+$ and $CD8^+$ T-cell effector function or activation status, as measured by stimulated interferon-γ production and CD69 expression when comparing post-pembrolizumab samples to baseline (Figure 2C). There were no differences between frequencies of effector memory and central memory T-cell subsets following pembrolizumab treatment in either relapsed or non-relapsed cohorts (*data not shown*). Aside from a decrease in PD-1 expression levels, which is likely the result of epitope masking by pembrolizumab binding to PD-1 on T cells,[10] there were no significant differences in the exhaustion marker TIM-3, or in the exhausted T-cell progenitor marker TCF-1, in either $CD4^+$ or $CD8^+$ T cells after pembrolizumab compared to baseline (*Online Supplementary Figure S2*).

Given the success of immune checkpoint inhibitors in solid tumors, and the accepted mechanism of action of allogeneic HSCT in which donor T cells eliminate residual leukemic cells through the graft-*versus*-leukemia effect, it follows that PD-1/PD-L1 inhibition could be an attractive target for

Table 1. Patients' characteristics.

Variable	Values
Age in years, mean (IQR)	68.5 (65.5-73.5)
Sex, N (%)	
Male	6 (50)
Female	6 (50)
Performance status, N (%)	
ECOG 0	4 (33)
ECOG 1	8 (66)
Type of AML, N (%)	
De novo	8 (66.6)
Secondary	4 (33.3)
ELN 2017 risk, N (%)	
Favorable	2 (16.7)
Intermediate	5 (41.6)
Adverse	5 (41.6)
Mutations, N (%)	
NPM1	6 (50)
FLT3-ITD	4 (33)
IDH1 or *IDH2*	6 (50)
TP53	2 (16.7)
WBC count at diagnosis, x10^9/L, mean (IQR)	2.3 (1.3-13.45)
Hemoglobin at diagnosis, g/dL, mean (IQR)	8.9 (7.2-11.25)
Platelet count at diagnosis, x10^9/L, mean (IQR)	73.5 (52.5-140)
% Blasts at diagnosis, mean (IQR)	67 (50-77.5)
CR status after induction therapy, N (%)	
CR*	8 (66.6)
CRi**	4 (33.3)
Induction therapy, N (%)	
"7+3" regimen	9 (75)
Fludarabine and cytarabine	1 (8.3)
Liposomal cytarabine and daunorubicin	1 (8.3)
Cytarabine and decitabine	1 (8.3)
Consolidation therapy, N (%)	
High-dose cytarabine (1-3 g/m^2)	9 (75)
None	3 (25)

*Defined by <5% blasts in a bone marrow aspirate with absolute neutrophil count >1.0x10^9/L and platelets >100x10^9/L. **Defined by complete remission with residual neutropenia (absolute neutrophil count <1.0x10^9/L) or thrombocytopenia (platelets <100x10^9/L). IQR: interquartile range; ECOG: Eastern Cooperative Oncology Group performance status; AML: acute myeloid leukemia, ELN: European LeukemiaNet; ITD: internal tandem duplication; WBC: white blood cell; CR: complete remission; CRi: complete remission with incomplete hematologic recovery.

preventing AML relapse. However, studies thus far have been negative. In a similar trial to ours which investigated the use of the PD-1 blocking antibody nivolumab as maintenance therapy in 15 AML patients who received at least one cycle of consolidation therapy, Reville *et al*. demonstrated a median recurrence-free survival of 8.48 months.[11] In that study, all patients were high-risk as defined by having secondary AML, presence of high-risk cytogenetics or a *FLT3*-ITD mutation, presence of MRD at the time of enrollment, or were in second CR regardless of disease characteristics. Collectively, this may explain these patients' overall poorer outcomes compared to ours.[11] Consistent with our findings, grade 3/4 immune-related adverse events were observed in 27% of the patients treated with nivolumab.[11] Separately, Liu *et al*. recently presented the results of a multicenter, open-label, randomized phase II study which assessed the efficacy of nivolumab maintenance therapy for patients with AML in first CR or CRi who were not candidates for allogeneic HSCT.[12] Patients who received nivolumab (N=38) were well matched to those randomized to observation alone (N=41), with a mean age of 63.2 and 65.5 years, respectively. Liu *et al*. did not observe significantly different outcomes between cohorts, as the median progression-free survival among patients treated with nivolumab was 13.2 months (95% confidence interval: 8.5-21.8 months) compared to 10.9 months for those on observation (95% confidence interval: 5.4-14.9 months, *P*=0.38), with a median overall survival of 53.9 months *versus* 30.9 months, respectively (*P*=0.23). There were more adverse events of any grade

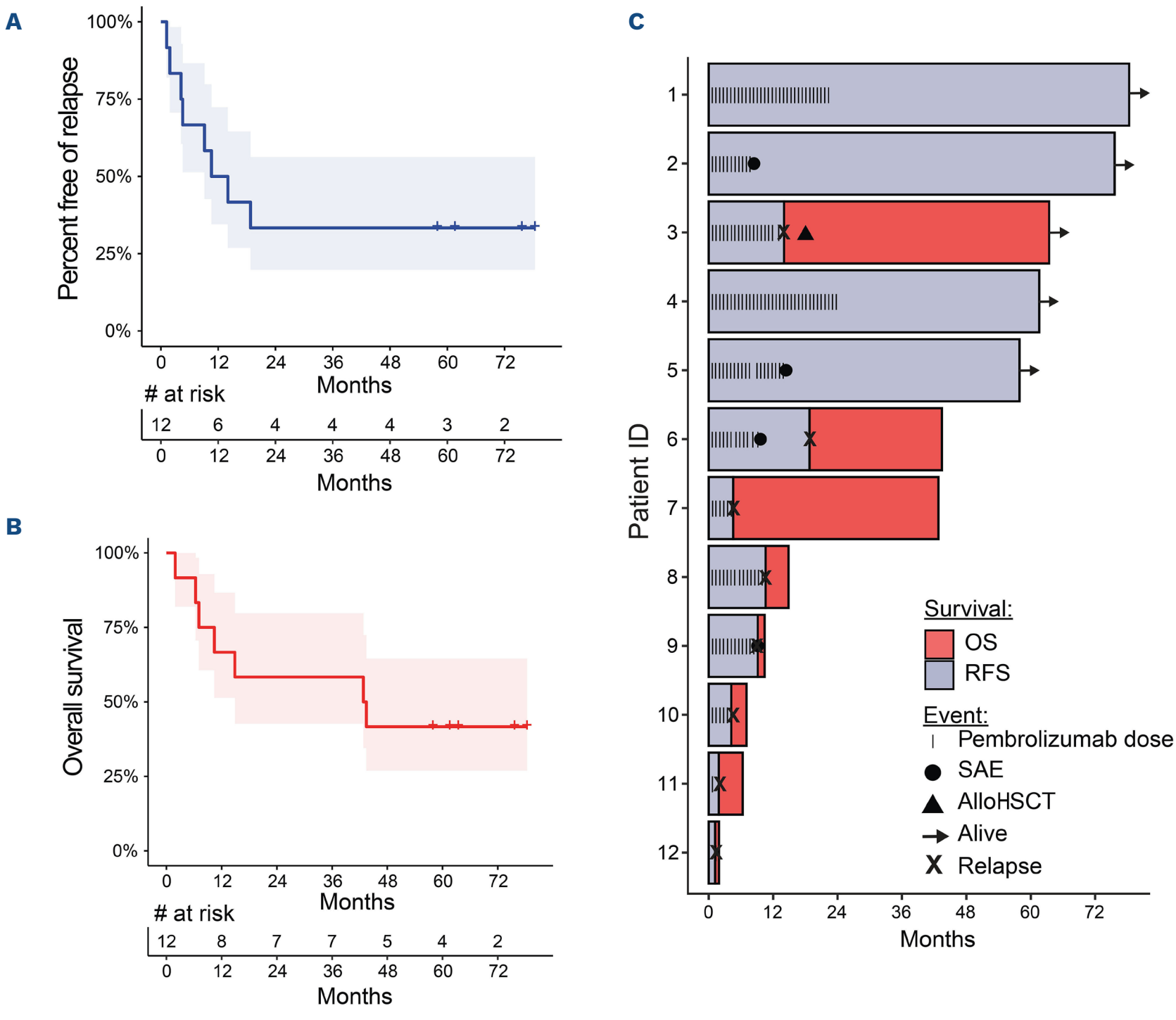

Figure 1. Patients' outcomes. (A, B) Kaplan-Meier curves showing the percentage of patients free from relapse (A) and overall survival (B) among 12 patients with acute myeloid leukemia in first complete remission treated with pembrolizumab. Shaded areas indicate 80% confidence intervals. (C) Swimmer plot showing relapse-free survival and overall survival of individual patients treated with pembrolizumab. ID: identity; OS: overall survival; RFS: relapse-free survival; SAE: serious adverse event; AlloHSCT: allogeneic hematopoietic stem cell transplantation.

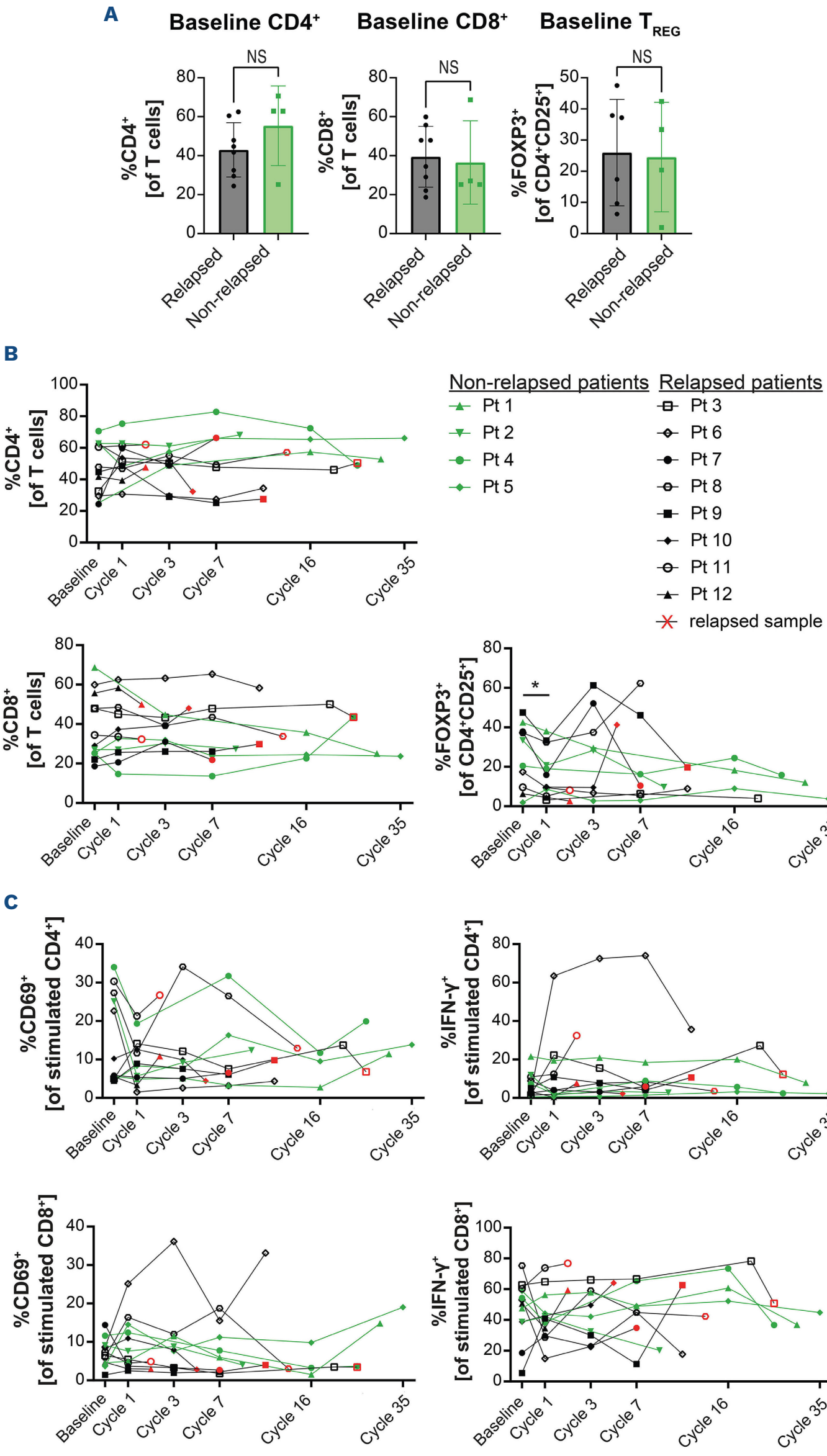

Figure 2. T-cell frequencies and function over time in peripheral blood. (A) Pre-treatment peripheral blood mononuclear cells were stained for surface CD3 (clone: OKT3), CD8 (clone: HIT8a), CD4 (clone: OKT4) CD25 (clone: M-A251, all from Biolegend, San Diego, CA, USA), and intracellular FOXP3 (clone: 206D, Cell Signaling, Danvers, MA, USA) using a FOXP3/transcription factor staining buffer (Thermo-Fisher, Waltham, MA, USA). The graphs show the frequencies of $CD4^+$, $CD8^+$ and $CD4^+CD25^+FOXP3^+$ regulatory T cells (T_{REG}) in peripheral blood stratified by patients who relapsed and those who did not. No significant differences in the T-cell subsets were observed between the two groups by two-sided unpaired *t* tests. (B) Individual frequencies of total, $CD4^+$, $CD8^+$ and $CD4^+CD25^+FOXP3^+$ T_{REG} cells were also assessed in peripheral blood at the end of pre-specified maintenance pembrolizumab cycles (1, 3, 7, 16, and 35), or at end of treatment or disease relapse. After the first cycle of pembrolizumab there was a transient decrease of T_{REG} (P=0.02 by a two-sided paired t test compared to the pre-treatment baseline control). No significant changes in the frequency of $CD4^+$ or $CD8^+$ T cells were observed following therapy. (C) To test T-cell function, peripheral blood mononuclear cells were stimulated with phorbol myristate acetate and ionomycin (Sigma Aldrich, St. Louis, MO, USA) for 6-8 hours followed by overnight incubation with brefeldin A (BD Biosciences, San Jose, CA, USA) and staining for CD69 (clone FN50, Biolegend) and intracellular interferon (IFN)-γ (clone 4S.B3, Cell Signaling). The graphs show the post-stimulation frequencies of $CD69^+$ and IFN-γ^+ $CD4^+$ (top row) and $CD8^+$ T cells (bottom row) over time by patient. The use of pembrolizumab did not have an effect on $CD4^+$ and $CD8^+$ T-cell effector function. NS: not significant.

among nivolumab-treated patients compared to controls (71.0% *vs*. 12.2%; *P*<0.001), although all were manageable.[12] In two randomized phase II studies comparing azacitidine with or without the PD-L1 inhibitor durvalumab in newly diagnosed AML and high-risk myelodysplastic syndromes, Zeidan and colleagues observed no improvement of response rates with the addition of durvalumab, regardless of disease mutation status.[13,14] The marginal clinical responses seen in these trials of immune checkpoint blockade in myeloid malignancies do not appear to be limited to the PD-1/PD-L1 axis either, as a recently published placebo-controlled randomized phase II trial testing the anti-TIM-3 antibody sabatolimab given with a hypomethylating agent in previously untreated high-risk myelodysplastic syndromes failed to meet its primary endpoints of improvements of complete response rates or progression-free survival.[15]

In retrospective analyses of peripheral blood taken from patients with advanced solid tumors, treatment with pembrolizumab has been associated with a reduction in circulating FOXP3$^+$ regulatory T cells compared to baseline.[16,17] However, that we only observed a transient decrease in the frequency of FOXP3$^+$ regulatory T cells among CD4$^+$CD25$^+$ cells after initiation of pembrolizumab may be more consistent with reports of pembrolizumab interfering with the differentiation of regulatory T cells, but not their stability.[18] Additionally, our findings that T-cell frequency, activation status and effector function were not affected by pembrolizumab corroborates those of Goswami *et al*., who also did not identify a clear signature between responders and non-responders by immunophenotyping and single-cell RNA sequencing T cells obtained from relapsed AML patients treated with pembrolizumab and decitabine.[8] The relatively weak immunophenotypic shifts we observed contrast with the development of immune-related adverse events in this cohort, which could argue against an immunological basis for these events. In this case a placebo-controlled arm would be a useful comparator. Alternatively, smaller clonal populations of T cells may be contributing to autoimmune pathology in an antigen-specific manner; however, our approach is not sophisticated enough to allow detection of these expanded populations in peripheral blood.

A major limitation of our study is the relatively small cohort size and lack of a control arm. While our cohort of older adults with AML is reflective of this population and demonstrated a similar time to relapse as expected from historical data, direct comparisons to other maintenance trials are limited, in part, due to the differences and heterogeneity of patients enrolled in them. Furthermore, our exploratory analysis of T-cell immunophenotypes from peripheral blood may not reflect anti-leukemic responses within the bone marrow, nor corroborate biomarkers of response to pembrolizumab derived from pre-treatment bone marrow samples in other studies.[8,9]

In summary, among older AML patients in their first CR, pembrolizumab maintenance therapy is feasible and, when used with consolidation therapy, can result in durable remissions in select patients. Observed toxicities were mostly hematologic in nature and expected given our pre-treated cohort. Any-grade immune-related adverse events, which occurred after ten cycles of pembrolizumab in four of five cases, were manageable and could be accepted in this historically difficult-to-treat population. Since most immune-related adverse events occurred after the administration of more than ten cycles of pembrolizumab, a reasonable strategy to potentially prevent these events in future studies may be to discontinue pembrolizumab if MRD-negative CR can be documented on serial bone marrow biopsy specimens. Considering the marginal responses of immune checkpoint inhibitors seen by others, whether given alone or with chemotherapy or hypomethylating agents, our findings here further underscore the need for improved immunotherapeutic strategies for preventing relapse in AML and reliable biomarkers to identify those most likely to respond to them. Along these lines, agents such as pembrolizumab may be of interest to augment the effects of T-cell-mediated therapies currently being explored in clinical trials, such as vaccines, bispecific T-cell engagers and adoptive T-cell therapies.

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Disclosures

MB is adjunct Professor of Medicine at the University of Pittsburgh and an employee of Genentech. None of the other authors have any conflicts of interest to disclose.

Contributions

MB and AS conceived, designed and conducted the study. TLW and DPN contributed to the design of the protocol. KQ, KL and DPN analyzed the data. AR, AI, RLR, KAD, MA, J-ZH, RF and JR contributed patients to the study. Y-MSH performed flow cytometric analysis. All authors contributed to the preparation of the manuscript and approved the final manuscript.

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Data-sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. Wei AH, Döhner H, Pocock C, et al. Oral azacitidine maintenance therapy for acute myeloid leukemia in first remission. N Engl J Med. 2020;383(26):2526-2537.
2. Kantarjian H, Kadia T, DiNardo C, et al. Acute myeloid leukemia: current progress and future directions. Blood Cancer J. 2021;11(2):41.
3. Sharma P, Goswami S, Raychaudhuri D, et al. Immune checkpoint therapy - current perspectives and future directions. Cell. 2023;186(8):1652-1669.
4. Zhou Q, Munger ME, Highfill SL, et al. Program death-1 signaling and regulatory T cells collaborate to resist the function of adoptively transferred cytotoxic T lymphocytes in advanced acute myeloid leukemia. Blood. 2010;116(14):2484-2493.
5. Williams P, Basu S, Garcia-Manero G, et al. The distribution of T-cell subsets and the expression of immune checkpoint receptors and ligands in patients with newly diagnosed and relapsed acute myeloid leukemia. Cancer. 2019;125(9):1470-1481.
6. Tang L, Wu J, Li C-G, et al. Characterization of immune dysfunction and identification of prognostic immune-related risk factors in acute myeloid leukemia. Clin Cancer Res. 2020;26(7):1763-1772.
7. Boyiadzis MM, Kirkwood JM, Marshall JL, Pritchard CC, Azad NS, Gulley JL. Significance and implications of FDA approval of pembrolizumab for biomarker-defined disease. J Immunother Cancer. 2018;6(1):35.
8. Goswami M, Gui G, Dillon LW, et al. Pembrolizumab and decitabine for refractory or relapsed acute myeloid leukemia. J Immunother Cancer. 2022;10(1):e003392.
9. Zeidner JF, Vincent BG, Ivanova A, et al. Phase II trial of pembrolizumab after high-dose cytarabine in relapsed/refractory acute myeloid leukemia. Blood Cancer Discov. 2021;2(6):616-629.
10. Pluim D, Ros W, Miedema IHC, Beijnen JH, Schellens JHM. Multiparameter flow cytometry assay for quantification of immune cell subsets, PD-1 expression levels and PD-1 receptor occupancy by nivolumab and pembrolizumab. Cytometry A. 2019;95(10):1053-1065.
11. Reville PK, Kantarjian HM, Ravandi F, et al. Nivolumab maintenance in high-risk acute myeloid leukemia patients: a single-arm, open-label, phase II study. Blood Cancer J. 2021;11(3):60.
12. Liu H, Sharon E, Karrison TG, et al. Randomized phase II study to assess the role of nivolumab as single agent to eliminate minimal residual disease and maintain remission in acute myelogenous leukemia (AML) patients after chemotherapy (NCI9706 protocol; REMAIN trial). Blood. 2022;140(Supplement 1):1716-1719.
13. Zeidan AM, Boss I, Beach CL, et al. A randomized phase 2 trial of azacitidine with or without durvalumab as first-line therapy for older patients with AML. Blood Adv. 2022;6(7):2219-2229.
14. Zeidan AM, Boss I, Beach CL, et al. A randomized phase 2 trial of azacitidine with or without durvalumab as first-line therapy for higher-risk myelodysplastic syndromes. Blood Adv. 2022;6(7):2207-2218.
15. Zeidan AM, Ando K, Rauzy O, et al. Sabatolimab plus hypomethylating agents in previously untreated patients with higher-risk myelodysplastic syndromes (STIMULUS-MDS1): a randomised, double-blind, placebo-controlled, phase 2 trial. Lancet Haematol. 2024;11(1):e38-e50.
16. Gambichler T, Schröter U, Höxtermann S, Susok L, Stockfleth E, Becker JC. Decline of programmed death-1-positive circulating T regulatory cells predicts more favourable clinical outcome of patients with melanoma under immune checkpoint blockade. Br J Dermatol. 2020;182(5):1214-1220.
17. Fameli A, Nardone V, Shekarkar Azgomi M, et al. PD-1/PD-L1 immune-checkpoint blockade induces immune effector cell modulation in metastatic non-small cell lung cancer patients: a single-cell flow cytometry approach. Front Oncol. 2022;12:911579.
18. Sasidharan Nair V, Toor SM, Taouk G, et al. Pembrolizumab interferes with the differentiation of human FOXP3+–induced T regulatory cells, but not with FOXP3 stability, through activation of mTOR. J Immunol. 2020;204(1):199-211.