haematologica
Journal of the Ferrata Storti Foundation

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

by Kevin Quann, Konstantinos Lontos, Alison Sehgal, Anastasios Raptis, Annie Im, Robert L. Redner, Kathleen A. Dorritie, Mounzer Agha, Jing-Zhou Hou, Rafic Farah, James Rossetti, Daniel P. Normolle, Theresa L. Whiteside, Yen-Michael Sheng Hsu, and Michael Boyiadzis

Received: Feb 18, 2024.
Accepted: July 31, 2024.

Citation: Kevin Quann, Konstantinos Lontos, Alison Sehgal, Anastasios Raptis, Annie Im, Robert L. Redner, Kathleen A. Dorritie, Mounzer Agha, Jing-Zhou Hou, Rafic Farah, James Rossetti, Daniel P. Normolle, Theresa L. Whiteside, Yen-Michael Sheng Hsu, and Michael Boyiadzis. A phase II study of post-remission therapy with pembrolizumab in older patients with acute myeloid leukemia.
Haematologica. 2024 Aug 8. doi: 10.3324/haematol.2024.285313 [Epub ahead of print]

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

Running Title: Post-remission pembrolizumab in older patients with AML

Kevin Quann[1,2*], Konstantinos Lontos[2], Alison Sehgal[2], Anastasios Raptis[2], Annie Im[1,2], Robert L. Redner[1,2], Kathleen A. Dorritie[1,2], Mounzer Agha[2], Jing-Zhou Hou[2], Rafic Farah[2], James Rossetti[2], Daniel P Normolle[3], Theresa L. Whiteside[2,4], Yen-Michael Sheng Hsu[5], and Michael Boyiadzis[1]

[1]Department of Medicine, Division of Hematology/Oncology, University of Pittsburgh School of Medicine, Pittsburgh PA

[2]UPMC Hillman Cancer Center, Pittsburgh, PA

[3]Department of Biostatistics, University of Pittsburgh School of Public Health, Pittsburgh, PA

[4]Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA

[5]Immunologic Monitoring and Cellular Products Laboratory, UPMC Hillman Caner Center, Pittsburgh, PA

*Corresponding author: Kevin Quann, MD, PhD

UPMC Cancer Pavilion

5150 Centre Avenue, 5th Floor

Pittsburgh, PA 15232

email: quannka@pitt.edu

KEYWORDS:

Acute myeloid leukemia, pembrolizumab, clinical trial, post-remission therapy, immune checkpoint inhibition, leukemia relapse

ClinicalTrials.gov ID: NCT02708641

ACKNOWLEDGEMENTS:

We would like to thank the patients and their families for participating in this trial.

FUNDING:

This trial was supported by funding from Merck. KQ was supported in part by the Hillman Postdoctoral Fellowship for Innovative Cancer Research. YMSH receives Hillman Senior Fellowship for Innovative Cancer Research and is supported by CCSG P30 CA047904. This work also utilized the Hillman Cancer Center Immunologic Monitoring and Cellular Products Laboratory, a shared resource at the University of Pittsburgh supported by the CCSG P30 CA047904.

AUTHOR CONTRIBUTIONS:

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

DECLARATION OF COMPETING INTEREST:

M.B is adjunct Professor of Medicine at the University of Pittsburgh and an employee of Genentech. All authors have no competing interests.

DATA AVAILABILITY STATEMENT:

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

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 (allo-HCT). In this population, hypomethylating agents, and in particular orally administered azacitidine, may be used as maintenance therapy to prolong remissions, with the latter receiving FDA approval after demonstrating an improvement in median relapse-free survival of over 5 months compared to 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 anti-tumor 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 and PD-L1 were upregulated in murine leukemia cells and antibody-mediated blockade of these proteins 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 that 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 hematological malignancies[7]. In AML, pembrolizumab has been investigated with decitabine, or as maintenance therapy for those achieving 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 post-remission could promote an anti-leukemia immune response and prevent or delay leukemia relapse. Here, we

conducted a single-arm phase 2 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 that achieved complete remission (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 wildtype *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 (ANC) $\geq 1.0\times10^9$/L and platelet count $\geq 50\times10^9$/L at time of enrollment. The study was approved by the University of Pittsburgh Institutional Review Board and all patients signed written informed consent (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 ELN 2017 criteria, both with mutated *NPM1* and wild-type *FLT3*, with mutated *DNMT3A*. Eight patients were in CR and 4 patients in CRi at enrollment. Measurable residual disease (MRD) by flow cytometry (sensitive to 1 in 10^4 cells) was evaluable from bone-marrow aspirate in 10 patients prior to starting pembrolizumab and all were MRD negative. Longitudinal MRD assessments were not performed during the study conduct. All patients were induced with intensive regimens, with 75% receiving 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, 3 patients (25%) did not receive consolidation chemotherapy before enrolling 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 to 35), with a median time to relapse (mTTR) of 12.3 months **(Figure 1A)** and a median overall survival (mOS) of 43.1 months **(Figure 1B).** Two patients completed 2 years of maintenance pembrolizumab and 7 discontinued therapy due to leukemia relapse. Five patients (41%) developed immune-related adverse events (irAEs) requiring interventions, of which 4 discontinued pembrolizumab. At time of analysis, no patients were continuing maintenance pembrolizumab. Among the 8 patients that relapsed, one underwent re-induction and allo-HCT, achieving a durable second CR, whereas the remaining relapsed patients died from their disease **(Figure 1C).** In subgroup analyses, mTTR was significantly longer among patients with ECOG 0 performance status at time of enrollment: 18.7 months vs 3.0 months (ECOG 1), p=0.00018. Median TTR of those who received consolidation therapy was also significantly improved compared to those who did not, 18.8 months vs 4.2 months, p=0.019 (**Figure S1A**). No relapses were observed among two patients with favorable risk by ELN criteria, whereas intermediate risk patients had a mTTR of 14.0 months and adverse risk patients had a mTTR of 4.2 months (p=0.034). Median overall survival was similarly correlated with performance status: mOS not reached (NR; ECOG 0) vs 6.7 months (ECOG 1), p=0.0032, receipt of consolidation therapy: mOS NR (received consolidation) vs 7.0 months (did not receive consolidation), p=0.007, and leukemia risk: mOS NR (favorable), NR (intermediate) and 7.0 months (adverse), p=0.011 (**Figure S1B**).

Among the irAEs 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 anti-viral therapy and discontinuation of pembrolizumab; one patient developed grade 4 immune thrombocytopenia after cycle 19 that responded to steroids and discontinuation of pembrolizumab; 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. **Supplemental Table 1** 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)**. Further, this significance was not reached 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 IFN-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 decreasing 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 nor exhausted T cell progenitor maker TCF-1, in either $CD4^+$ or $CD8^+$ T cells post-pembrolizumab compared to baseline (**Figure S2**).

Given the success of immune checkpoint inhibitors in solid tumors, and the accepted mechanism of action of allo-HCT in which donor T cells eliminate residual leukemic cells through the graft-vs-leukemia effect, it follows that PD-1/PD-L1 inhibition could be an attractive target for 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 time of enrollment, or were in second CR regardless of disease characteristics. Collectively, this may explain their overall poorer patient outcomes compared to our results[11]. Consistent with our findings, grade 3/4 immune-related adverse events were observed in 27% of patients treated with nivolumab[11]. Separately, Liu et al recently presented the results of a multi-center, 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 allo-HCT[12]. Patients that 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. They did not observe significantly different outcomes between cohorts, as the median progression-free survival among patients treated with nivolumab was 13.2 months (95% CI 8.5-21.8 months) compared to 10.9 months for those on observation (95% CI 5.4 – 14.9

months, p=0.38), with a median overall survival of 53.9 months versus 30.9 months (p=0.23). There were more adverse events of any grade among nivolumab-treated patients compared to controls (71.0% vs 12.2%; p <0.001), though all were manageable[12]. In two randomized phase 2 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 addition of durvalumab, regardless of disease mutation status[13,14]. The marginal clinical responses seen from 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 2 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 $FOXP3^+$ T_{REG} among $CD4^+CD25^+$ cells after initiation of pembrolizumab may be more consistent with reports of pembrolizumab interfering with differentiation of T_{REG} 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 irAEs 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, yet 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 patient 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 irAEs, which occurred after 10 cycles of pembrolizumab in 4 of 5 cases, were manageable and could be accepted in this historically difficult to treat population. Since most irAEs occurred after the administration of more than 10 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.

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.

Table 1: Patient characteristics.

Variable	N (%)
Age (mean, IQR**)**	68.5 (65.5 – 73.5)
Sex	
Male	6 (50)
Female	6 (50)
Performance status	
ECOG 0	4 (33%)
ECOG 1	8 (66%)
De Novo vs Secondary AML	
De novo	8 (66.6)
Secondary	4 (33.3)
ELN 2017 risk	
Favorable	2 (16.7)
Intermediate	5 (41.6)
Adverse	5 (41.6)
Mutations	
NPM1	6 (50)
FLT3-ITD	4 (33)
IDH1 or *IDH2*	6 (50)
TP53	2 (16.7)
White blood cell count at diagnosis, x 10^9/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, x 10^9/L (mean, IQR)	73.5 (52.5 – 140)
% Blasts at diagnosis (mean, IQR)	67 (0.5 – 0.775)
CR status after induction therapy*	
CR	8 (66.6)
CRi	4 (33.3)
Induction therapy	
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	
Hi-dose Cytarabine (1-3 g/m^2)	9 (75)
None	3 (25)

*CR: complete remission as defined by < 5% blasts in bone marrow aspirate with ANC > 1.0 x 10^9/L and platelets > 100 x10^9/L; CRi: complete remission with incomplete hematologic as defined by residual neutropenia (ANC < 1.0 x 10^9/L) or thrombocytopenia (platelets < 100 x10^9/L).

FIGURE LEGENDS:

Figure 1: Patient outcomes. (**A**) Kaplan-Meier curve showing percentage of patients free from relapse and (**B**) overall survival of twelve patients with AML in first complete remission treated with pembrolizumab. Shaded areas indicate 80% confidence intervals. (**C**) Swimmer's plot showing relapse free survival (RFS) and overall survival (OS) of individual patients treated with pembrolizumab.

Figure 2: T cell frequencies and function over time in peripheral blood. (**A**) Pre-treatment PBMCs were stained for surface CD3 (clone: OKT3), CD8 (clone: HIT8a), CD4 (clone: OKT4) CD25 (clone: M-A251, all from Biolegend, San Diego, CA), and intracellular FOXP3 (clone: 206D, Cell Signaling, Danvers, MA) using a FOXP3/transcription factor staining buffer (Thermo-Fisher, Waltham, MA). Shown are 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 2 groups by two-sided unpaired t-test. (**B**) Individual frequencies of total, $CD4^+$, $CD8^+$ and $CD4^+CD25^+FOXP3^+$ regulatory T 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 regulatory T cells ($p=0.02$ by two-sided paired t-test compared to 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, PBMCs were stimulated with phorbol myristate acetate (PMA) and ionomycin (Sigma Aldrich, St. Louis, MO) for 6-8 hours followed by overnight incubation with Brefeldin A (BD Biosciences, San Jose, CA) and staining for CD69 (clone FN50, Biolegend) and intracellular IFN-γ (clone 4S.B3, Cell Signaling). Shown are 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 the $CD4^+$ and $CD8^+$ T-cell effector function.

Figure 1

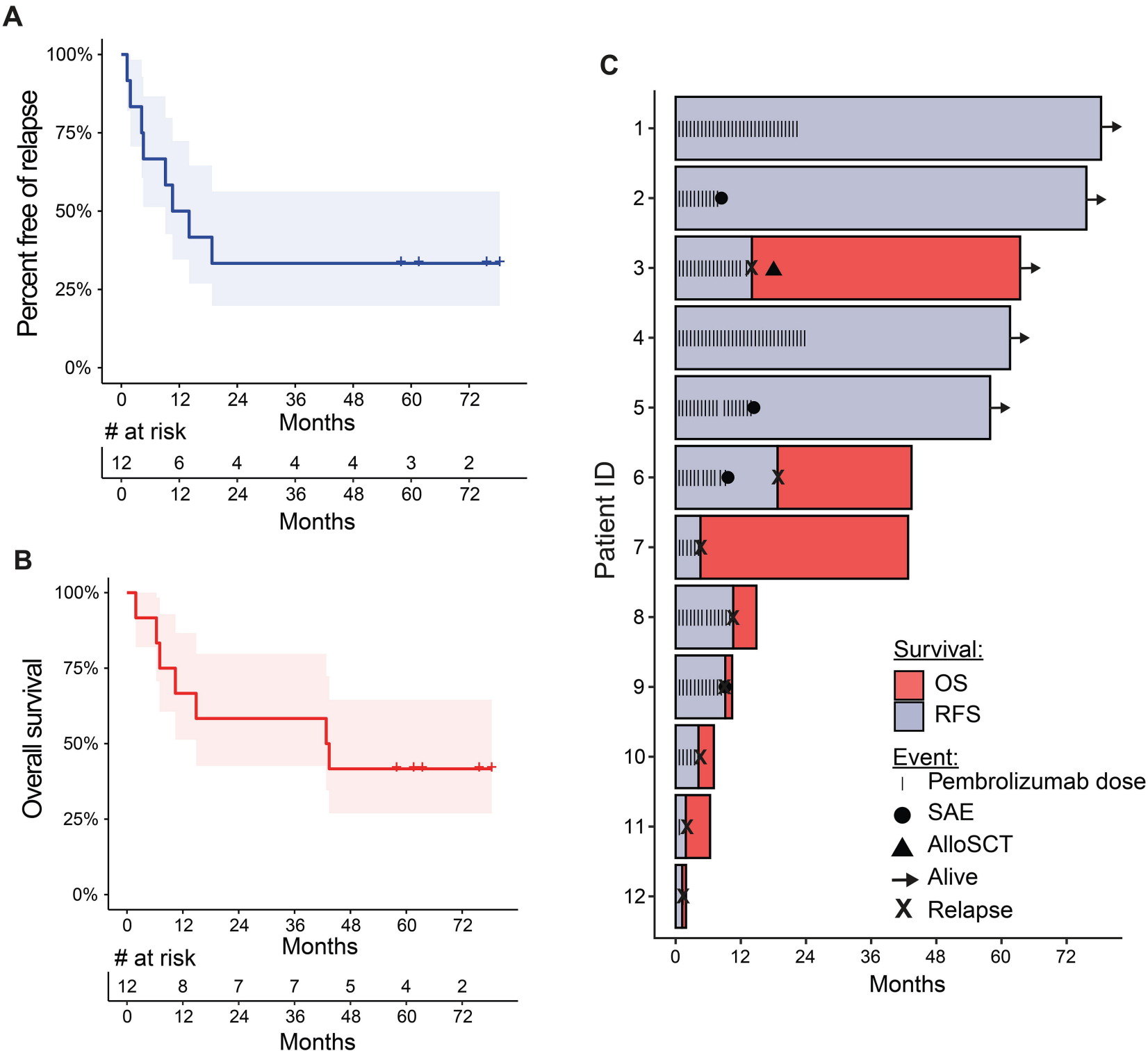
A
100%
75%
50%
25%
0%
Percent free of relapse
0
12
24
36
48
60
72
Months
at risk
12
6
4
4
4
3
2
0
12
24
36
48
60
72
Months
B
100%
75%
50%
25%
0%
Overall survival
0
12
24
36
48
60
72
Months
at risk
12
8
7
7
5
4
2
0
12
24
36
48
60
72
Months
C
Patient ID
1
2
3
4
5
6
7
8
9
10
11
12
0
12
24
36
48
60
72
Months
Survival:
OS
RFS
Event:
Pembrolizumab dose
SAE
AlloSCT
Alive
Relapse

Figure 2

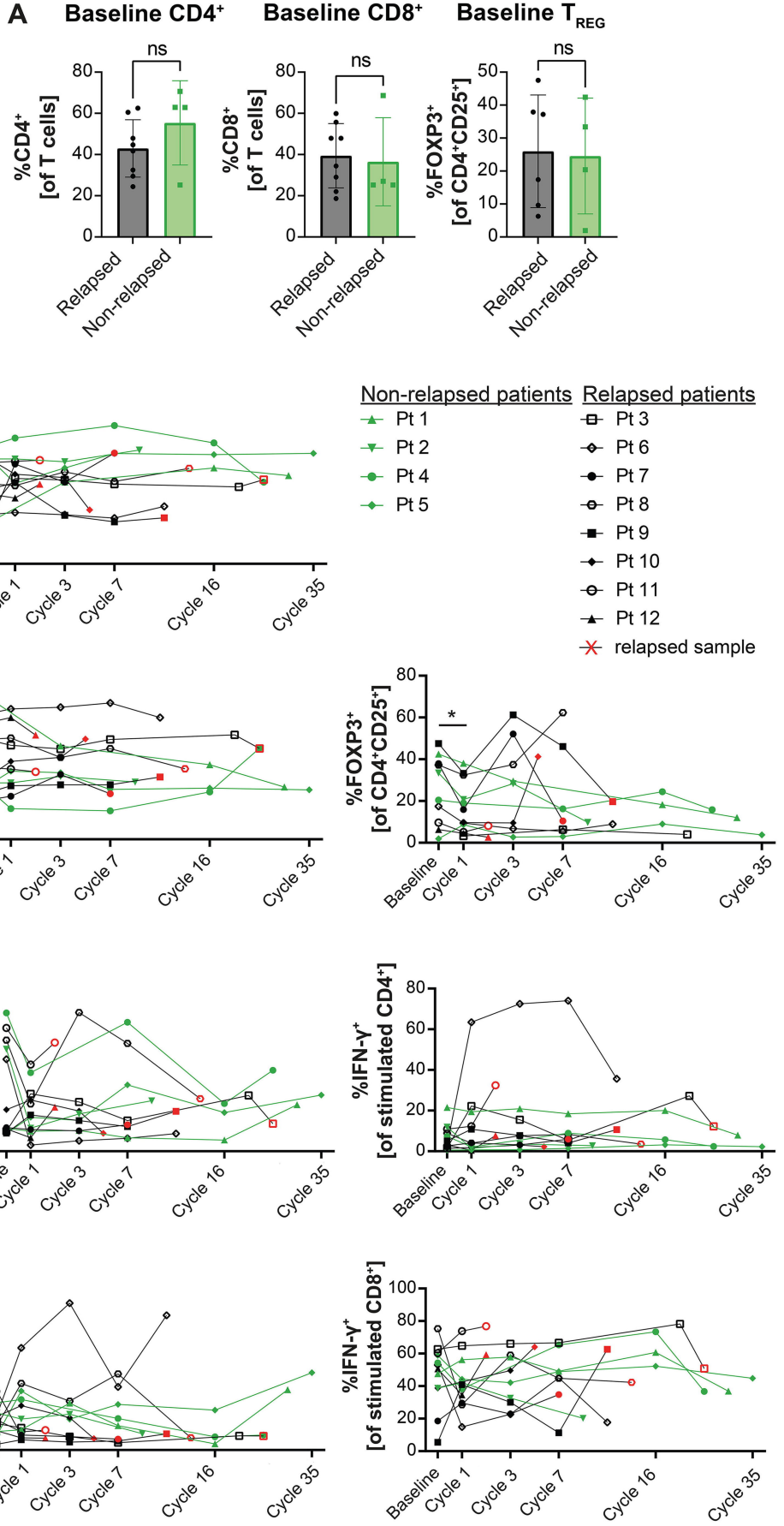
A
Baseline CD4+
Baseline CD8+
Baseline TREG
%CD4+ [of T cells]
%CD8+ [of T cells]
%FOXP3+ [of CD4+CD25+]
ns
Relapsed
Non-relapsed
B
Non-relapsed patients
Pt 1
Pt 2
Pt 4
Pt 5
Relapsed patients
Pt 3
Pt 6
Pt 7
Pt 8
Pt 9
Pt 10
Pt 11
Pt 12
relapsed sample
*
Baseline
Cycle 1
Cycle 3
Cycle 7
Cycle 16
Cycle 35
C
%CD69+ [of stimulated CD4+]
%IFN-γ+ [of stimulated CD4+]
%CD69+ [of stimulated CD8+]
%IFN-γ+ [of stimulated CD8+]

Figure S1

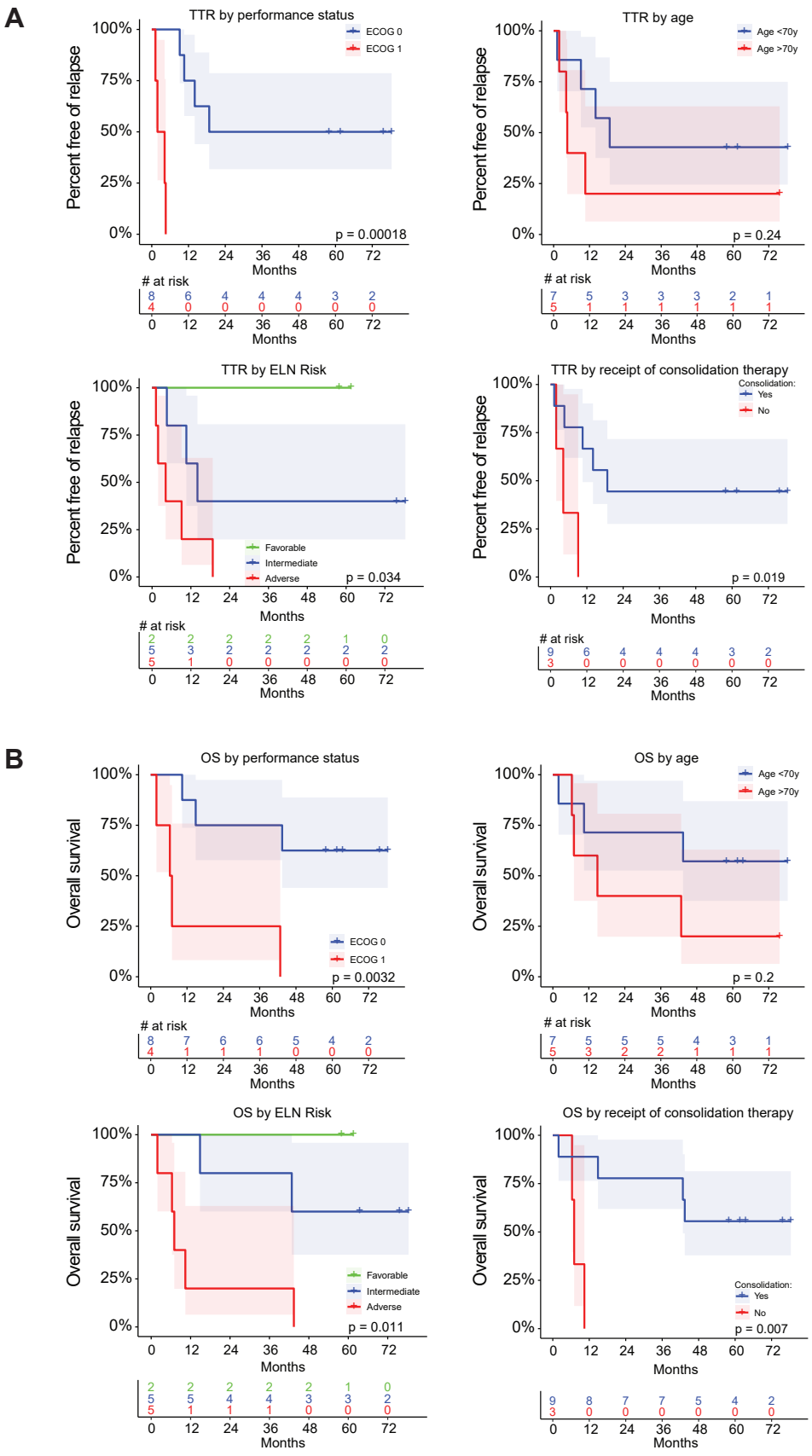

Figure S1: Kaplan Meier curves demonstrating relapse-free survival (**A**) and overall survival (**B**) according to subgroup by performance status, age, ELN risk stratification, and receipt of consolidation therapy. P-values between groups were computed by log-rank test.

Supplemental Table 1: Adverse events

	Grade I	Grade II	Grade III	Grade IV	Total	Grade III-IV
Platelet count decreased	5	0	2	3	10	5
Neutrophil count decreased	0	4	1	3	8	4
Anemia	3	2	1	0	6	1
Lymphocyte count decreased	0	2	3	0	5	3
Hyponatremia	4	0	1	0	5	1
Fatigue	1	4	0	0	5	0
Fever	2	2	0	0	4	0
Diarrhea	2	0	1	0	3	1
Hypocalcemia	1	1	0	0	2	0
Hypokalemia	1	1	0	0	2	0
Hypomagnesemia	2	0	0	0	2	0
Hypophosphatemia	2	0	0	0	2	0
Hypothyroidism	1	1	0	0	2	0
Creatinine increased	2	0	0	0	2	0
Pruritus	1	1	0	0	2	0
Aspartate aminotransferase increased	0	0	1	0	1	1
Alkaline phosphatase increased	1	0	0	0	1	0
Hyperglycemia	1	0	0	0	1	0
Hyperkalemia	1	0	0	0	1	0
Hypoalbuminemia	1	0	0	0	1	0
Hypoglycemia	1	0	0	0	1	0
Anorexia	1	0	0	0	1	0
Blurred vision	1	0	0	0	1	0
Chills	1	0	0	0	1	0
Cough	1	0	0	0	1	0
Dizziness	1	0	0	0	1	0
Edema limbs	1	0	0	0	1	0
Headache	0	1	0	0	1	0
Hematuria	0	0	1	0	1	1
Hypertension	0	0	1	0	1	1
Hypotension	0	1	0	0	1	0
Lung infection	0	1	0	0	1	0
Pneumonitis	0	1	0	0	1	0
Sore throat	1	0	0	0	1	0
Urinary tract infection	0	1	0	0	1	0

Figure S2

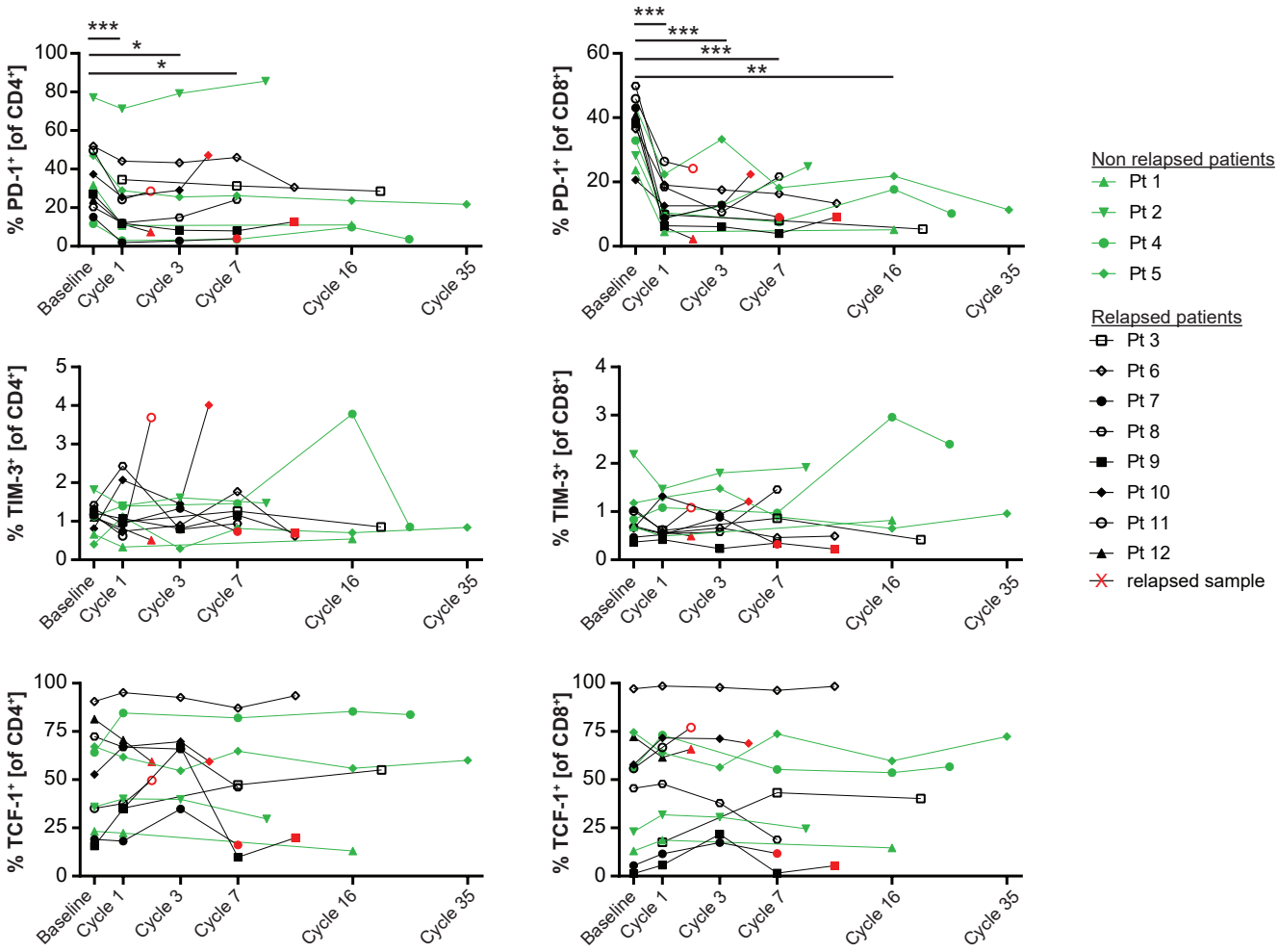

Figure S2: PBMCs were immediately isolated from peripheral blood using Ficoll-Hypaque (Sigma Aldrich, St. Louis, MO) gradient centrifugation and cryopreserved. At time of flow cytometric analysis, PBMCs were thawed and resuspended in RPMI-1640 with 10% fetal bovine serum, 1% non-essential amino acids, 1 mM sodium pyruvate, 1% Glutamax and 10 mM HEPES (all from Thermo-Fisher, Waltham, MA) for culture and staining. Cells were stained for surface CD3 (clone: OKT3), CD4 (clone OKT4), CD8 (clone HIT8a), PD1 (clone EH12.2H7) and TIM-3 (clone F38-2E2, all from Biolegend, San Diego, CA). Intracellular staining for TCF-1 (clone C63D9, Cell Signaling, Danvers, MA) was performed using a FOXP3/transcription factor staining kit (Thermo-Fisher). A live/dead fixable dye (Biolegend) was added to exclude dead cells. Samples were acquired on an BD LSRFortessa flow cytometer (BD Biosciences, San Jose, CA) and analyzed using FlowJo software. The analysis gates were restricted to the lymphocytes based on forward and side scatter, followed by live T cells excluding viability dye that were $CD3^+$, then $CD4^+$ and $CD8^+$ cells, on which $PD\text{-}1^+$, $TIM\text{-}3^+$, and $TCF\text{-}1^+$ subpopulations were gated. Shown are frequencies of $PD\text{-}1^+$, $TIM\text{-}3^+$, and $TCF\text{-}1^+$ populations among CD4+ (left column) and CD8+ T cells (right column) at the end of pre-specified maintenance pembrolizumab cycles (1, 3, 7, 16, and 35), or at end of treatment or disease relapse. *p<0.05, **p<0.01, and ***p<0.001 by two-sided paired t-test compared to pre-treatment baseline control.