Journal for ImmunoTherapy of Cancer

Three-year survival, correlates and salvage therapies in patients receiving first-line pembrolizumab for advanced Merkel cell carcinoma

Paul Nghiem ,[1] Shailender Bhatia ,[1] Evan J Lipson ,[2] William H Sharfman,[2] Ragini R Kudchadkar,[3] Andrew S Brohl,[4] Philip A Friedlander,[5] Adil Daud,[6] Harriet M Kluger,[7] Sunil A Reddy,[8] Brian C Boulmay,[9] Adam Riker,[9,10,11] Melissa A Burgess,[12] Brent A Hanks,[13] Thomas Olencki,[14] Kari Kendra,[14] Candice Church,[15] Tomoko Akaike ,[15] Nirasha Ramchurren,[16] Michi M Shinohara,[15] Bob Salim,[17] Janis M Taube,[2] Erin Jensen,[18] Mizuho Kalabis,[18] Steven P Fling,[16] Blanca Homet Moreno,[18] Elad Sharon ,[19] Martin A Cheever,[16] Suzanne L Topalian[2]

To cite: Nghiem P, Bhatia S, Lipson EJ, *et al*. Three-year survival, correlates and salvage therapies in patients receiving first-line pembrolizumab for advanced Merkel cell carcinoma. *Journal for ImmunoTherapy of Cancer* 2021;**9**:e002478. doi:10.1136/jitc-2021-002478

► Additional material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/jitc-2021-002478).

MAC and SLT contributed equally.

Accepted 16 March 2021

For numbered affiliations see end of article.

Correspondence to
Dr Paul Nghiem;
pnghiem@uw.edu

ABSTRACT

Background Merkel cell carcinoma (MCC) is an aggressive skin cancer associated with poor survival. Programmed cell death-1 (PD-1) pathway inhibitors have shown high rates of durable tumor regression compared with chemotherapy for MCC. The current study was undertaken to assess baseline and on-treatment factors associated with MCC regression and 3-year survival, and to explore the effects of salvage therapies in patients experiencing initial non-response or tumor progression after response or stable disease following first-line pembrolizumab therapy on Cancer Immunotherapy Trials Network-09/KEYNOTE-017.

Methods In this multicenter phase II trial, 50 patients with advanced unresectable MCC received pembrolizumab 2 mg/kg every 3 weeks for ≤2 years. Patients were followed for a median of 31.8 months.

Results Overall response rate to pembrolizumab was 58% (complete response 30%+partial response 28%; 95% CI 43.2 to 71.8). Among 29 responders, the median response duration was not reached (NR) at 3 years (range 1.0+ to 51.8+ months). Median progression-free survival (PFS) was 16.8 months (95% CI 4.6 to 43.4) and the 3-year PFS was 39.1%. Median OS was NR; the 3-year OS was 59.4% for all patients and 89.5% for responders. Baseline Eastern Cooperative Oncology Group performance status of 0, greater per cent tumor reduction, completion of 2 years of treatment and low neutrophil-to-lymphocyte ratio were associated with response and longer survival. Among patients with initial disease progression or those who developed progression after response or stable disease, some had extended survival with subsequent treatments including chemotherapies and immunotherapies.

Conclusions This study represents the longest available follow-up from any first-line anti-programmed death-(ligand) 1 (anti-PD-(L)1) therapy in MCC, confirming durable PFS and OS in a proportion of patients. After initial tumor progression or relapse following response, some patients receiving salvage therapies survived. Improving the management of anti-PD-(L)1-refractory MCC remains a challenge and a high priority.

Trial registration number NCT02267603.

BACKGROUND

Merkel cell carcinoma (MCC) is an aggressive neuroendocrine skin cancer that frequently spreads to nodal and distant sites. Prior to the use of immunotherapies targeting programmed cell death-1 (PD-1) or its major ligand programmed death-ligand 1 (PD-L1), patients with advanced MCC (aMCC) had an expected 5-year overall survival (OS) of 14%–27%.[1] The incidence of MCC is increasing mainly due to an aging population, with nearly 3000 cases in the USA this year.[2] MCC is an immunogenic cancer, with a higher incidence and poorer prognosis in immunosuppressed individuals.[3–6] Evidence of active immunity within and near the tumor has been described; notably, cell surface expression of PD-L1 by tumor cells and by tumor infiltrating lymphocytes is present in 49% and 55% of specimens, respectively.[7] Approximately 80% of MCCs are caused by the Merkel cell polyomavirus (MCPyV).[8] Virus-positive tumors (VP-MCC) persistently express T-antigen oncoproteins required for tumor cell proliferation, which are recognizable by the immune system as indicated by detection of MCPyV-specific T cells in peripheral blood and tumors from most patients with VP-MCC.[9] Furthermore, MCPyV-specific T cells often have high expression of PD-1 and Tim-3 on their surface indicating evidence

of potentially reversable immune dysfunction.[10] The remaining ~20% of MCCs are caused by ultraviolet light (UV) exposure (MCPyV-negative or VN-MCC). VN-MCCs contain abundant UV-induced mutations potentially generating neoantigens for immune recognition; their aggregate mutational burden is nearly 100-fold higher than that of VP-MCC tumors.[11–14]

Just a few years ago, standard-of-care treatment for aMCC was cytotoxic chemotherapy, which induced tumor regressions in ~60% of cases. However, responses to chemotherapy were not durable,[15] with a median progression-free survival (PFS) of only ~90 days. More recently, several clinical trials of PD-1 pathway inhibitors in patients with aMCC demonstrated improved PFS and OS compared with historical data for conventional cytotoxic chemotherapy. Favorable outcomes from these trials supported US Food and Drug Administration approvals for avelumab (Bevencio, anti-PD-L1) in March 2017 and pembrolizumab (Keytruda, anti-PD-1) in December 2018. Response rates achieved in the first-line treatment setting were 50%–60%; unlike results from chemotherapy, these responses had greater durability.[16–18] Across all anti-PD-(L)1 trials in aMCC, response rates appeared similar regardless of tumor viral status, suggesting that tumor antigens in both VP-MCC and VN-MCC can serve as effective targets for tumor elimination by the immune system. These outcomes led to rapid changes in the National Comprehensive Cancer Network guidelines for treating aMCC, and anti-PD-(L)1 agents are now included as preferred first-line systemic therapies.[19]

The current study was undertaken to further characterize long-term outcomes and explore factors associated with survival after first-line anti-PD-1 therapy in aMCC. Here, we report findings from the phase II Cancer Immunotherapy Trials Network (CITN)-09/KEYNOTE-017 trial of pembrolizumab. This report represents the longest available follow-up for any first-line anti-PD-(L)1 therapy in aMCC, with a median period of 31.8 months. Furthermore, we investigated survival in individuals who manifested primary or acquired resistance to first-line anti-PD-1 therapy and received subsequent treatments, in an effort to devise improved therapeutic strategies for these patients.

METHODS

Patients

Patients with aMCC (distant metastatic or locoregional disease) not amenable to definitive surgery or radiation therapy, and measurable per Response Evaluation Criteria in Solid Tumors RECIST v1.1, were enrolled. Patients who had prior systemic therapy for MCC were excluded, with the exception of adjuvant chemotherapy if completed >6 months prior to initiating study treatment. More detailed patient eligibility criteria have been reported previously.[20] An initial cohort of 26 patients was enrolled between January and December 2015, with results reported in 2016.[20] The protocol was then amended to include 24 additional patients enrolled between March 2016 and May 2017, and preliminary results were reported with a median follow-up of 14.9 months.[16] Potential financial conflicts of the investigators were reported and managed according to institutional policies at each center.

Study design

The CITN-09/KEYNOTE-017 trial is a phase II, open-label, non-randomized Simon two-stage multicenter study. Per the Simon two-stage design for efficacy estimation, at least one response among the first group of nine treated patients was required in order to enroll additional patients. Patients received pembrolizumab 2 mg/kg intravenously every 3 weeks. Treatment continued for up to 2 years, or until the development of unacceptable adverse event(s) (AEs), progressive disease (PD), a complete response (CR) with at least 24 weeks of therapy and at least two treatments beyond the date of confirmed CR, consent withdrawal or physician discretion. Patients were followed for AEs, PFS, OS and treatments received after discontinuing the study drug.

Study objectives

The primary objective of the CITN-09/KEYNOTE-017 trial was to determine the clinical efficacy of systemic first-line therapy for aMCC with pembrolizumab (Keytruda/MK-3475). The primary end point was overall response rate (ORR) measured by RECIST V.1.1, defined as CR+partial response (PR). Secondary end points included PFS, duration of response (DOR) and OS. The study also collected data on subsequent treatments received by patients who had primary or acquired resistance to pembrolizumab. Exploratory objectives were to determine associations between clinical outcomes and baseline and on-treatment patient and tumor characteristics, including tumor viral status and PD-L1 expression.

Disease assessment

CT scans were performed at screening, 12 weeks after treatment initiation and at 9-week intervals thereafter as previously described.[20] Patients who appeared to have PD were allowed to continue to the next cycle of therapy if they were asymptomatic, had Eastern Cooperative Oncology Group performance status (ECOG PS) ≤1 and had no evidence of rapid tumor progression; patients were evaluated 4 weeks later to assess possible further progression. After 1 year of treatment, the CT scan frequency was decreased to 12-week intervals. RECIST V.1.1 evaluations of scans were initially conducted at the investigator/institutional level, followed by central radiological review.

Specimen acquisition

Pretreatment fresh or archival tumor biopsy samples (formalin-fixed paraffin-embedded) were obtained from all patients. Blood samples were collected at the time of radiographic studies.

Laboratory assessments

Patients were determined to have MCPyV-positive tumors if they produced small T-antigen-specific serum antibodies[21] or manifested large T-antigen expression in tumor biopsies via immunohistochemistry.[22] PD-L1 staining (anti-PD-L1 clone 22C3, Merck & Co, Kenilworth, New Jersey, USA) was performed at QualTek Molecular Laboratories on pretreatment tumor specimens as previously described.[17] Specimens were considered PD-L1 positive if ≥1% of tumor cells expressed PD-L1 at the cell surface.[16 20]

Neutrophil-to-lymphocyte ratio (NLR) was calculated using absolute neutrophil counts and absolute lymphocyte counts (ALC), determined from automated complete blood counts in peripheral blood specimens obtained at study visits. NLR was calculated at baseline (before initial pembrolizumab infusion) and after each of the first four treatment cycles.

Statistical analysis

All statistical analyses were based on a database cut-off date of October 23, 2019. Responses were evaluated with point estimates and 95% CIs based on the exact binomial method. Median DOR (for patients who had a CR or PR), PFS and OS with 95% CIs were estimated by the Kaplan-Meier (KM) method for censored data. For DOR, subjects who had not progressed by the last disease assessment were censored at the date of last disease assessment. For PFS, subjects without documented PD/death were censored at the last disease assessment date. Any subject who was lost to follow-up was included in the analysis, and their PFS time was censored on the last date the subject was known to be progression-free, defined as the date of the last tumor assessment not indicating progression. For OS, subjects without documented death at the time of data cut-off were censored at the date last known to be alive. Post hoc analyses of the relationships between baseline patient and tumor characteristics and survival time were also conducted using KM methods. HRs and corresponding 95% CIs were estimated. NLR and ALC analyses were performed with a mixed model approach. Briefly, each time point used a t-test allowing for unequal variance. P values for trends across all time points were based on mixed model, with treatment cycle and response status (CR/PR vs SD/PD) or survival status (alive vs dead) as fixed effects, with a random intercept.

RESULTS

Patient and treatment characteristics

Fifty patients with aMCC were enrolled between January 2015 and May 2017. Data were analyzed as of October 23, 2019, representing ≥30 months since treatment initiation for all patients. For those who received pembrolizumab continuously for the maximum treatment period of 2 years, the follow-up period included ≥6 months after completing treatment. Median follow-up at the time of analysis was 31.8 months (range 0.4–56.9). Baseline patient and tumor characteristics have been detailed previously.[16] Briefly, 43 (86%) patients had stage IV MCC and 7 (14%) had stage IIIB MCC[23] at the time of enrollment, and all subjects had an ECOG PS of 0 or 1.[24] Their median age was 70.5 years (range 46–91), similar to other studies of aMCC. Patients received a median of 10.5 doses of pembrolizumab (SD 12.7 doses; range 1–35). Twelve patients (24%) completed 2 years of treatment. Thirty-seven patients did not complete 2 years of therapy due to PD (n=19), AE (n=13), death (n=2), physician decision (n=2) or consent withdrawal (n=1). One patient was lost to follow-up; see online supplemental table S1.

Table 1 Summary of best response by blinded independent central review per Response Evaluation Criteria in Solid Tumors V.1.1

Response evaluation*	No. patients (n=50)	%	95% CI†
Complete response (CR)	15	30	17.9 to 44.6
Partial response (PR)	14	28	16.2 to 42.5
Objective response (CR+PR)	29	58	43.2 to 71.8
Stable disease (SD)	4	8	2.2 to 19.2
Disease control (CR+PR+SD)	33	66	51.2 to 78.8
Progressive disease	16	32	19.5 to 46.7
No assessment‡	1	2	0.1 to 10.6

*Only confirmed responses are included.
†Based on binomial exact CI method.
‡One subject had a baseline tumor assessment but could not be reassessed after starting therapy, due to illness and death before the first on-treatment scan.

Response and duration of response

Similar to the ORR of 56% reported earlier in this study,[16 20] with longer treatment and follow-up the ORR to pembrolizumab was 58% (95% CI 43.2 to 71.8); this included 15 patients with CR and 14 with PR (table 1). Among a total of 29 responders, the median response duration was not reached (NR, range 1.0+ to 51.8+ months; figure 1). At 3 years after treatment initiation, 72.7% of responders remained in response. Most objective tumor regressions occurred soon after treatment initiation, with 90% (26/29) of CRs and PRs documented at the initial ~12-week assessment (figure 2A,B).

Progression-free survival and overall survival

PFS and OS estimates for first-line pembrolizumab therapy in aMCC are shown in figure 3. The median PFS was 16.8 months (95% CI 4.6 to 43.4), and the KM estimate of PFS at 3 years was 39.1% (figure 3A). The median OS was not reached at the time of analysis (95% CI 26 months, not estimable). Notably, while the KM estimate of OS at 3 years was 59.4% for all patients, it was 89.5% for responders (CR+PR; figure 3B).

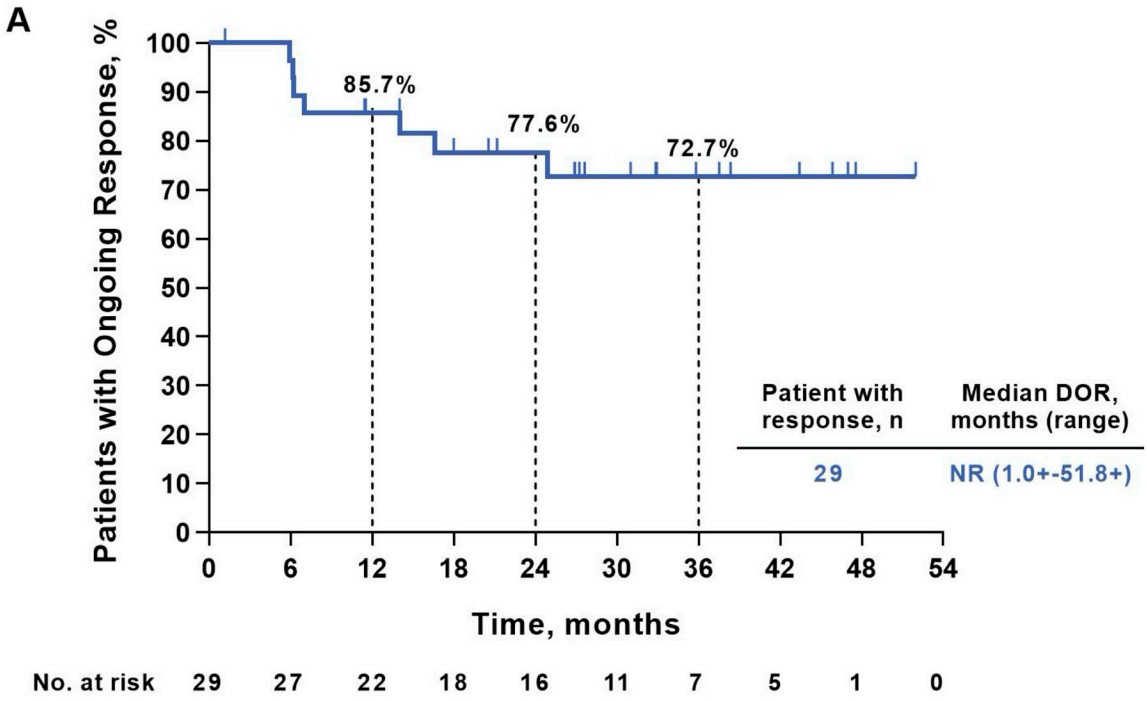


Figure 1 Duration of response (DOR). Kaplan-Meier curve showing duration of response among 29 patients having a complete or partial tumor regression by Response Evaluation Criteria in Solid Tumors V.1.1. Patients without an event were censored (tick mark) at the last disease assessment date. Rates of ongoing response at 12, 24 and 36 months are indicated. NR, not reached.

Factors associated with response and overall survival

Based on outcomes reported for anti-PD-1 therapies in some other cancer types,[25 26] we first asked if the degree of tumor burden reduction in patients with aMCC receiving pembrolizumab was associated with OS. Forty-five patients with evaluable tumor target lesions per RECIST V.1.1 were included in this analysis (figure 4). An increasing degree of tumor target lesion reduction was associated with prolonged OS, such that the majority of patients with 100% reductions survived for 30 months and beyond. These findings are consistent with the OS results shown in figure 3B, in which patients experiencing an objective response (CR+PR) to pembrolizumab therapy survived longer than the overall treatment population. Associations of several baseline patient and tumor features with OS were also assessed (figure 5 and online supplemental figure S1A). Patients who were able to complete 2 years of continuous pembrolizumab therapy were more likely to be alive with 30 months' follow-up (HR 0.1; 95% CI 0.01 to 0.73), while a baseline ECOG PS of 1 vs 0 was associated with a decreased likelihood of survival (HR 2.7; 95% CI 1.10 to 6.64). Interestingly, the magnitude of baseline tumor burden (above or below the median, figure 5; or absolute dimensions, online supplemental figure S1A) was not associated with OS, nor were age (< vs ≥70 years), gender, anatomic sites of metastases, or tumor viral or PD-L1 status (figure 5). Analysis of the same factors with objective response did not yield any significant associations (online supplemental table S2 and figure S1B).

Cell counts in the peripheral blood at baseline and during pembrolizumab treatment were also assessed for potential correlations with objective response and OS. When trends were assessed across the first 3 months of therapy, the NLR but not the ALC was associated with objective response (CR+PR, p=0.043) and OS (p=0.028) at 30 months (online supplemental figure S2). Specifically, a lower NLR across all time points was associated with improved outcomes. However, the results of similar assessments conducted at baseline only, or at any individual time point during therapy, were not statistically significant.

Adverse events

AEs experienced by patients in this study are summarized in online supplemental table S3. Treatment-related adverse events (TRAEs) of any grade occurred in 49 of 50 patients (98%), and 15 patients (30%) had grade ≥3 TRAEs, similar to earlier reported results from this trial.[16] In the setting of longer treatment duration, eight patients (16%) discontinued treatment due to TRAEs, similar to seven patients (14%) reported earlier. A single treatment-related death occurred and was detailed previously.[16] These results suggest that TRAEs were not cumulative with prolonged anti-PD-1 therapy for aMCC, as previously shown for patients with other cancer types receiving anti-PD-1 continuously for up to 2 years.[27] Immune-mediated TRAEs and infusion reactions occurred in 16 patients (32%) (summarized in online supplemental table S4).

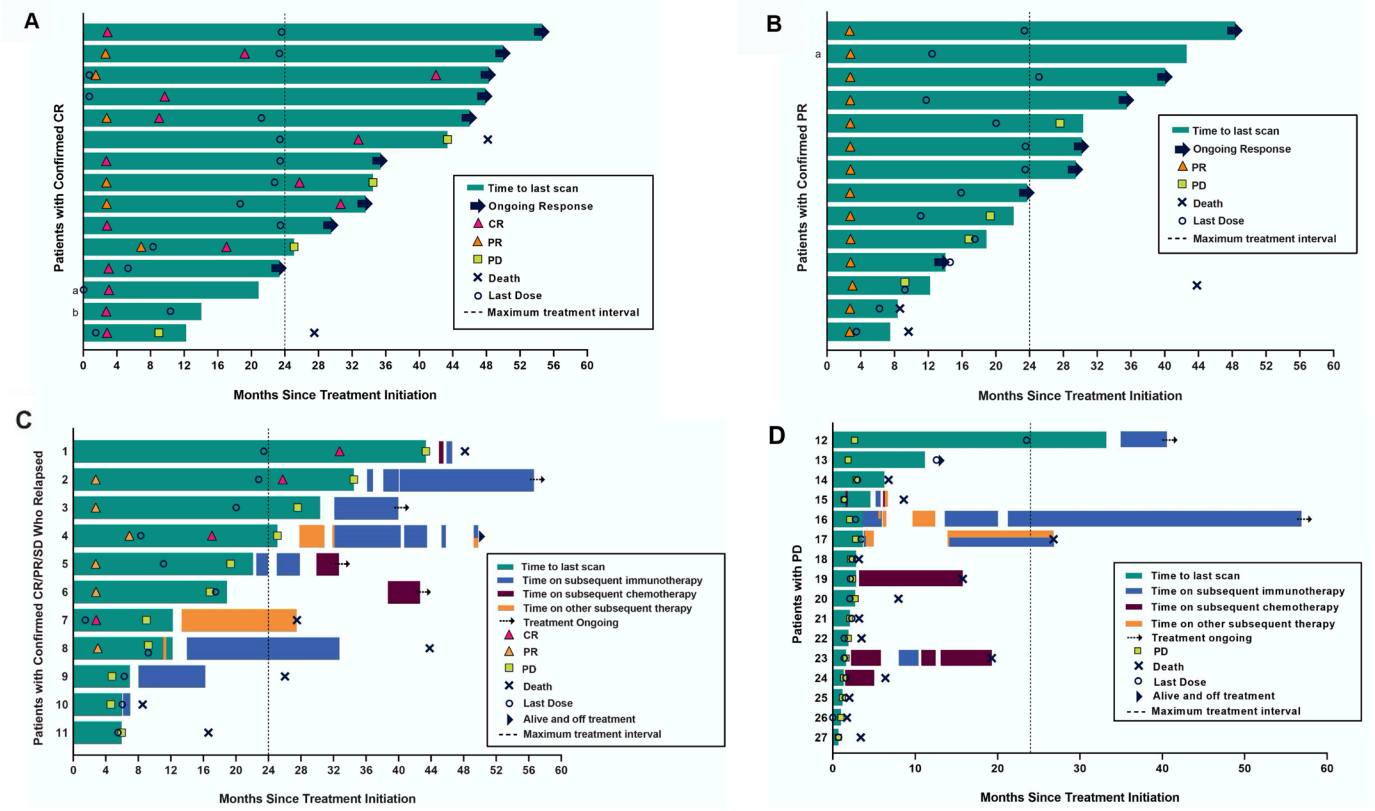


Figure 2 Kinetics of response to pembrolizumab, and subsequent treatments received by patients with tumor relapse or with no response. Each lane in these swimmer plots depicts an individual patient. Dotted vertical lines indicate the maximum on-study pembrolizumab treatment interval (24 months). (A) Patients with a confirmed complete response (CR) to pembrolizumab (n=15). [a,b]Two patients were censored for progression/response because they started a new anticancer therapy without documented disease progression. (B) Patients with a confirmed partial response (PR) to pembrolizumab therapy (n=14). [a]This patient was censored for progression/response because they started a new anticancer therapy without documented disease progression. (C) Patients with CR (red triangle), PR (yellow triangle) or stable disease (SD) (patients #9, 10, 11) after receiving pembrolizumab on-study, who later experienced disease progression (n=11). Subsequent treatments are shown. Patients with CR or PR are also depicted in panels (A) and (B), respectively. Details of subsequent treatments are presented in online supplemental table S6. (D) Patients with initial progressive disease (PD) (no CR, PR or SD) on pembrolizumab (n=16), showing subsequent treatments received. Details of subsequent treatments are presented in online supplemental table S6.

Salvage therapies for anti-PD-1-resistant aMCC

Currently, available data describing effective subsequent therapies for patients with cancer who experience primary or acquired resistance to anti-PD-(L)1 therapy are limited. To gain insights into potentially effective therapeutic options for patients with anti-PD-1-refractory aMCC, we collected subsequent treatment data from those who received pembrolizumab on the CITN-09/KEYNOTE-017 trial. In total, there were 22 patients who received other therapies for MCC after discontinuing on-study pembrolizumab, including a variety of chemotherapies, immunotherapies and experimental treatments (listed in online supplemental table S5).

Eleven patients depicted in figure 2C developed resistance to pembrolizumab after an initial response (CR, n=4; PR, n=4) or SD (n=3; patients #9–11 as shown); for the eight patients with CR or PR, the time interval between first response and disease progression varied widely. Among these 11 patients, 10 received additional therapies; 8 received subsequent immunotherapies, including pembrolizumab, nivolumab, avelumab, ipilimumab and combination nivolmab+ipiliumumab. Five of the 10 (50%) patients with subsequent therapies were alive at the time of data analysis, 4 of whom had received immunotherapies. Eight of 10 (80%) patients with initial CR/PR/SD who relapsed and received subsequent therapies survived for >12 months after disease progression was documented on-study.

There were 16 patients with primary resistance to pembrolizumab (figure 2D). Among them, seven received subsequent therapies, while others expired soon after developing PD. Five of 16 (38%) patients survived >12 months after disease progression on-study, all having received subsequent treatment(s). Three patients were alive at the time of data analysis. Details of treatments received on a per-patient basis are shown in online supplemental table 6).

DISCUSSION

This multi-institutional study provides the longest available follow-up for first-line anti-PD-(L)1 therapy in

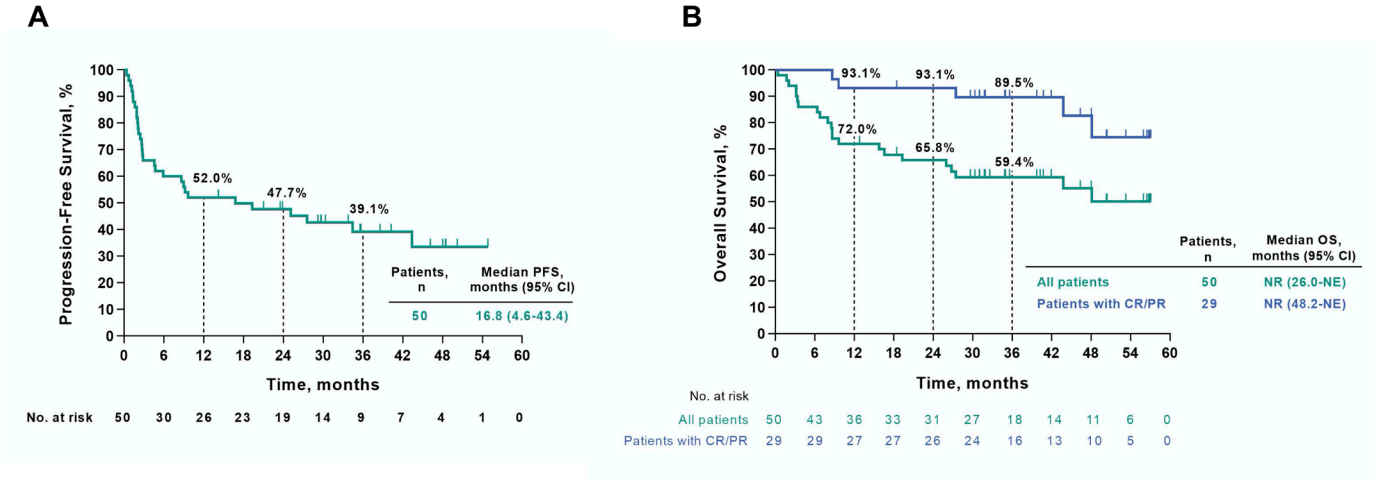


Figure 3 Survival among patients with advanced Merkel cell carcinoma (aMCC) receiving pembrolizumab. (A) Progression-free survival (PFS). Kaplan-Meier curve depicting PFS measured from the time of treatment initiation until either disease progression (Response Evaluation Criteria in Solid Tumors V.1.1) or death, whichever occurred first. At 36 months, the estimated PFS was 39.1%. Median PFS was 16.8 months (95% CI 4.6 to 43.4). (B) Overall survival (OS). Kaplan-Meier curves depicting OS among all 50 patients in green, or among those with objective tumor regression (complete response (CR)+partial response (PR)) in blue. At 36 months, the estimated OS was 59.4% for all patients, and 89.5% for those with objective response. Median OS was not reached in either group at the time of analysis. NR, not reached.

advanced unresectable MCC. All 50 patients were assessed ≥30 months following treatment initiation, with a median follow-up of 31.8 months. After a potential maximum continuous treatment period of 2 years, the ORR of 58% was very similar to earlier reports from this trial (56%). This likely reflects the rapid kinetics of anti-PD-1 response in MCC, with most responses occurring at the first radiographic evaluation (12 weeks).[16 20] With prolonged follow-up, the majority of responses were durable: 73% persisted at 3 years, and the median DOR was not reached. Furthermore, the median OS for all patients in this study was not reached. Importantly, objective

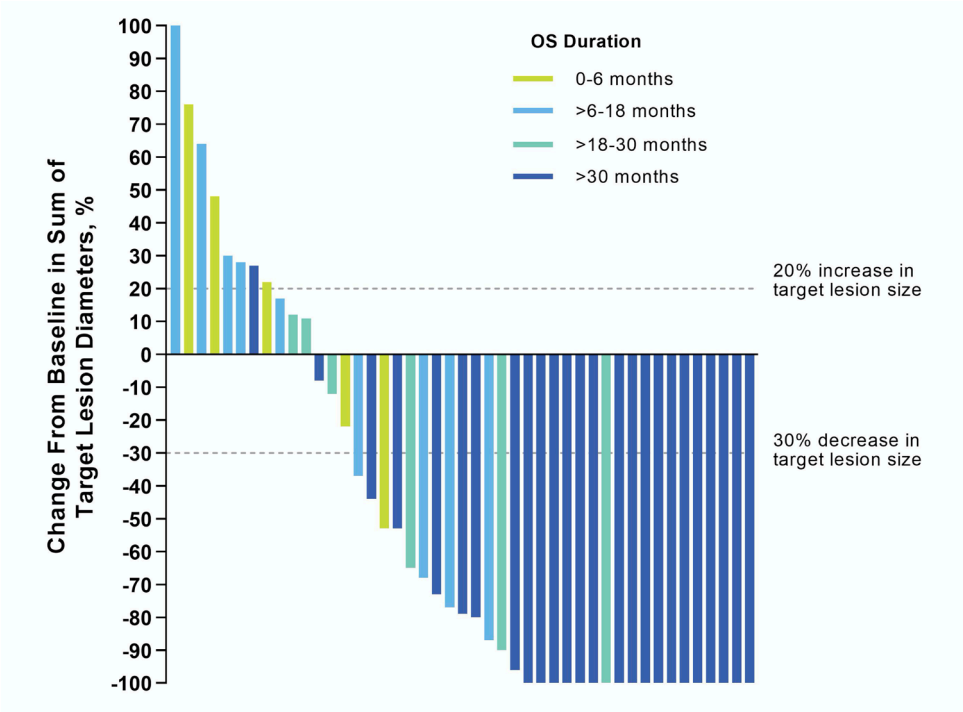


Figure 4 Association between magnitude of tumor burden reduction and overall survival (OS). Waterfall plot showing the maximum change in tumor burden (sum of target lesion diameters) compared with baseline, for radiographically evaluable patients (n=45). Horizontal dashed lines indicate Response Evaluation Criteria in Solid Tumors V.1.1 criteria for partial response (≥30% decrease in sum of target lesion diameters from baseline, in the absence of new lesions) and progressive disease (≥20% increase in sum of target lesion diameters). Vertical bars are color-coded to indicate OS duration in individual patients.

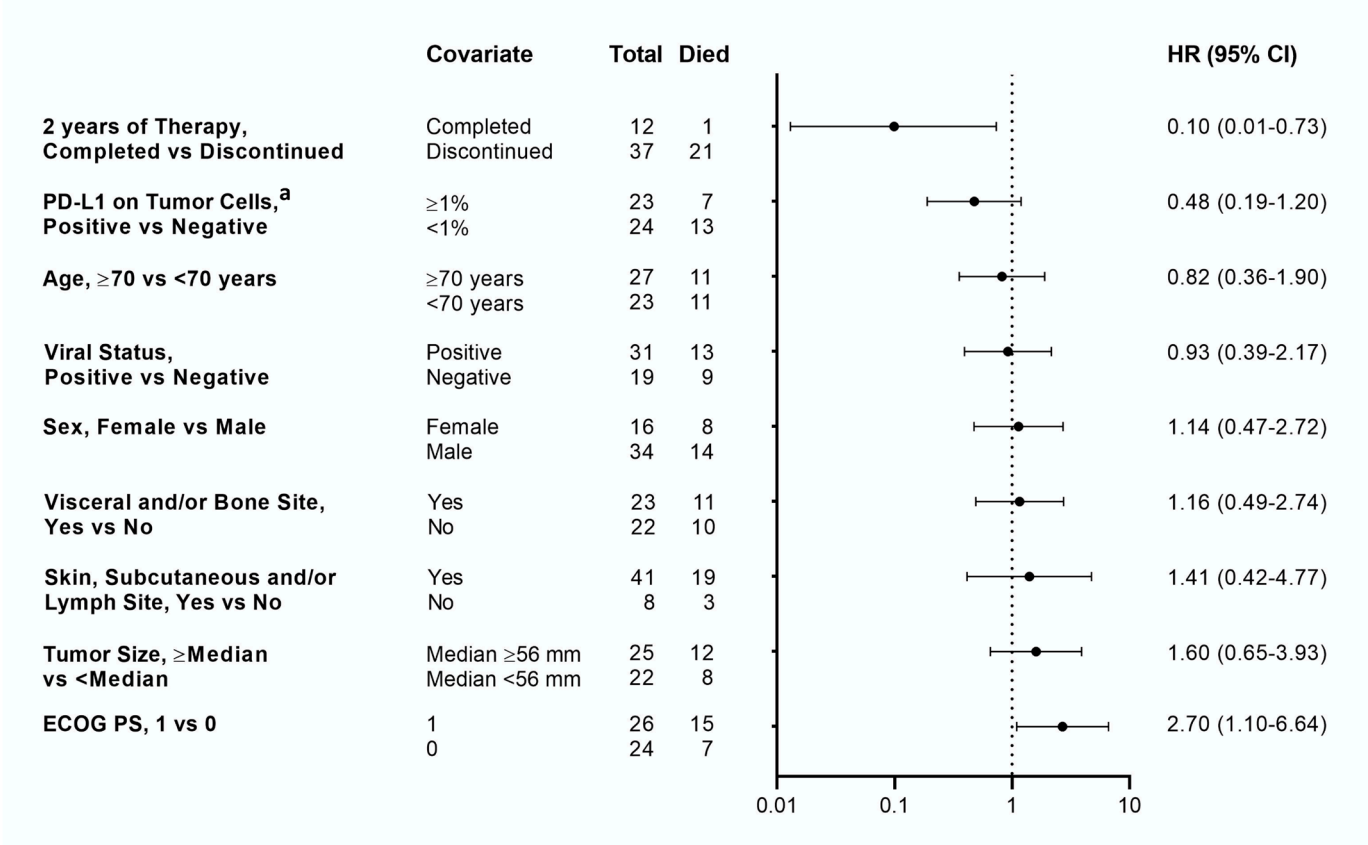


Figure 5 Association of overall survival with 30 months' follow-up, with baseline demographics and tumor and treatment characteristics. Forest plot showing overall survival HRs (with 95% CI) for characteristics which are listed from top to bottom in increasing order of HR magnitude. Total numbers of evaluable patients in each category are shown. Patients with baseline Eastern Cooperative Oncology Group performance status (ECOG PS) of 1 vs 0 had significantly reduced survival, while those who completed 2 years of pembrolizumab therapy experienced significantly longer survival. programmed death-ligand 1 (PD-L1) positive, ≥1% of tumor cells expressed cell surface PD-L1, assessed by immunohistochemistry.

responders had a substantially improved OS (89.5%) compared with the total study population (59.4%) at 3 years, suggesting that objective response is an early predictor of long-term survival in patients with aMCC receiving first-line anti-PD-1 therapy. Similarly, in studies of anti-PD-1 therapy in patients with advanced melanoma, non-small-cell lung cancer or renal cell carcinoma, objective responses correlated with long-term OS.[25 26] In the current MCC study in which 86% of patients had stage IV disease,[16] regardless of response status, the median OS far exceeded the 9.6-month median survival anticipated for patients with a new diagnosis of distant metastatic MCC before the advent of anti-PD-(L)1 therapies.[28] These findings of high response rate and durability, associated with extended survival, supported regulatory approval of pembrolizumab for aMCC based on non-randomized data and underline the enormous impact that anti-PD-(L)1 therapy has had on the outlook for patients with aMCC.

Here, we identify several baseline and on-treatment factors associated with survival assessed 30 months after initiating first-line pembrolizumab therapy for aMCC: ECOG PS 0, greater magnitude of reduction in tumor burden, and successful completion of 2 years of continuous therapy. Conversely, we also identified factors not associated with OS, including age, gender, baseline tumor burden, anatomic sites of metastasis and tumor PD-L1 expression and viral status. While baseline ECOG PS, magnitude of tumor burden reduction and duration of continuous anti-PD-1 administration[29] have been associated with response and survival in studies of anti-PD-1 therapy for various cancer types, the lack of association of several other factors as reported here for aMCC diverges from prior experience.[25 30 31] This may reflect an extremely robust response to anti-PD-1 therapy in highly immunogenic MCCs that can override the influence of other demographic or on-treatment factors. Although the current study permitted a maximum continuous treatment period of 2 years, it is unknown if this is sufficient or optimal for aMCC, or if treatment duration should be individualized depending on anti-PD-(L)1 response status. This important issue has been examined in a randomized trial in non-small-cell lung cancer,[29] which demonstrated survival benefit from continuous anti-PD-1 vs discontinuing at 1 year; this remains to be explored in MCC and other cancers.

Interestingly, our study also associated low peripheral blood NLR over the treatment course with objective tumor response and survival. A prognostic association

between high baseline blood NLR and decreased OS has been reported for several different cancer types,[32 33] and specifically for MCC.[34] Furthermore, in the context of anti-PD-(L)1 therapy, high baseline and/or on-treatment NLRs have been reported to predict OR and OS in melanoma, non-small-cell lung cancer, renal cell carcinoma and other cancers.[35–38] In the current study of first-line pembrolizumab for aMCC in immunocompetent patients, most ALCs were within the normal range and ALC as a single factor was not associated with response or survival, suggesting the importance of blood neutrophils as potentialy reflecting immune-suppressive inflammation, which might be driven by tumor-secreted IL-8 or other neutrophil-stimulating factors.[39]

The immunotherapy field is currently challenged with managing primary anti-PD-(L)1 resistance or relapse after an initial response (acquired resistance).[40] Improving the management of anti-PD-(L)1-refractory MCC remains a high priority. Our study describes salvage treatments received by these patients. Several initial responders with subsequent relapse had sustained survival after retreatment with immune checkpoint blockade, similar to published experience in other cancers.[41–44] However, among those with primary anti-PD-1 resistance, many expired soon after disease progression, although a few patients derived sustained survival from subsequent immunotherapies or chemotherapies. Beyond available immunotherapies and chemotherapies for advanced MCC, innovative clinical trial development is needed to address or prevent anti-PD-(L)1-refractory disease. Diverse approaches to address this problem include the addition of anti-CTLA-4 to anti-PD-(L)1,[45] toll-like receptor agonists,[46] histone deacetylase inhibitors[47] and oncolytic virotherapy.[48] In particular, infusion of MCPyV-specific T cells combined with immune checkpoint inhibitors may reduce the chance of tumor escape by boosting T cell numbers, increasing diversity of T cell responses and augmenting terminally exhausted T cells (NCT03747484). A therapeutic vaccine targeting MCPyV antigens is also an appealing approach to prevent recurrent disease as well as potentially overcome PD-(L)1 pathway resistance.[49] Both adjuvant and neoadjuvant anti-PD-(L)1 immunotherapies hold promise for preventing high-risk early stage resectable MCC from advancing to stage IV.[50]

Author affiliations
[1]University of Washington / Fred Hutchinson Cancer Research Center, Seattle, Washington, USA
[2]Johns Hopkins Bloomberg~Kimmel Institute for Cancer Immunotherapy and Kimmel Cancer Center, Baltimore, Maryland, USA
[3]Emory University, Atlanta, Georgia, USA
[4]Moffitt Cancer Center, Tampa, Florida, USA
[5]Mount Sinai Medical Center, New York, New York, USA
[6]University of California San Francisco, San Francisco, California, USA
[7]Yale University, New Haven, Connecticut, USA
[8]Stanford University, Stanford, California, USA
[9]Louisiana State University, New Orleans, Louisiana, USA
[10]Department of Surgery, Anne Arundel Medical Center, Annapolis, Maryland, USA
[11]DeCesaris Cancer Institute, Cancer Service Line, Luminis Health, Parole, Maryland, USA
[12]University of Pittsburgh, Pittsburgh, Pennsylvania, USA
[13]Duke University Medical Center, Durham, North Carolina, USA
[14]Ohio State University Comprehensive Cancer Center, Columbus, Ohio, USA
[15]University of Washington, Seattle, Washington, USA
[16]Fred Hutchinson Cancer Research Center / Cancer Immunotherapy Trials Network, Seattle, Washington, USA
[17]Axio Research, LLC, Seattle, Washington, USA
[18]Merck & Co Inc, Kenilworth, New Jersey, USA
[19]National Cancer Institute, Cancer Therapy Evaluation Program, Bethesda, Maryland, USA

Twitter Paul Nghiem @PaulNghiem, Evan J Lipson @evanlipson, Ragini R Kudchadkar @ragikudchadkar, Adil Daud @ad1600, Michi M Shinohara @michi_shinohara and Elad Sharon @EladSharonMD

Acknowledgements The authors would like to thank the patients and their families and caregivers for participating in this study. The authors acknowledge the efforts of the Cancer Immunotherapy Trials Network (CITN) across the participating clinical sites in conducting this trial, and Judith Kaiser for budgetary and regulatory oversight of CITN; Sumia Dakhil, University of Washington/Fred Hutchinson Cancer Research Center, Seattle, USA, for clinical trial support; Trish Brothers, Uchechi Nwaba and Katrina Purtell, Johns Hopkins Kimmel Cancer Center and Bloomberg~Kimmel Institute for Cancer Immunotherapy, Baltimore, USA for clinical trial support; and Elliot K. Chartash, Steven M. Townson, Scot Ebbinghaus, Scott Diede, Rachel Lewis, Jenia Booth, Diana Yurewicz, Steven R. Bird and Nageatte Ibrahim, Merck & Co., Inc., Kenilworth, New Jersey, USA, for collaboration. The authors would also like to thank Doyel Mitra, ApotheCom, Yardley, Pennsylvania, USA, for editorial assistance; this assistance was funded by Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, New Jersey, USA in accordance with Good Publications Practice guidelines.

Contributors PN, SB, RK, HK, JT, ES, MC, SLT were involved in the conception, design and planning of the study. PN, SB, EL, WHS, RK, ASB, PF, AD, HK, SR, BCB, AR, MB, BAH, TO, KK, CC, NR, MMS and JT collected data. PN, CC, TA, BS, JT, EJ, MK, SPF, BHM, ES, MC and SLT analyzed and interpreted data. PN, CC, TA and SLT drafted the manuscript and critically reviewed and revised the manuscript for important intellectual content with contributions from all authors. All authors reviewed the manuscript and agreed with its content and submission.

Funding Supported by the National Cancer Institute (NCI) Grants No. 1U01CA154967 (MC), P01 CA225517 (PN), R01 CA142779 (SLT and JT) and the National Institutes of Health/NCI Cancer Center Support Grant in Seattle Grant No. P30 CA015704. This study was also funded by the Merkel cell carcinoma (MCC) patient gift fund at the University of Washington, the Kelsey Dickson MCC Challenge Grant from the Prostate Cancer Foundation and Merck & Co., Inc., Kenilworth, New Jersey, USA, which provided pembrolizumab and partial funding for this study.

Competing interests PN reports grants from Bristol-Myers Squibb and EMD-Serono; advisory fees from EMD-Serono, Pfizer and Merck & Co.; travel expenses from Sanofi/Regeneron and Merck & Co. and has a pending patent related to high-affinity T-cell receptors that target the Merkel polyomavirus. SB reports personal fees from Bristol-Myers Squibb, EMD-Serono and personal fees and other from Sanofi-Genzyme; grants from Bristol-Myers Squibb, EMD-Serono, Merck & Co., NantKwest, Novartis, Immune Design, Oncosec, Exicure, Nektar; and personal fees from Castle Biosciences. EJ reports grants from Merck & Co., Bristol-Myers Squibb and Sanofi/Regeneron; personal fees from Bristol-Myers Squibb, Novartis, Array BioPharma, Macrogenics, Sanofi/Regeneron and Genentech. RK reports grants from Merck & Co., Bristol-Myers Squibb and Regeneron; advisory fees from Merck & Co., Bristol-Myers Squibb, Regeneron, Novartis and Array. ASB reports personal fees from Bayer, Deciphera and EMD Serono. BAH reports grants from Merck & Co., Tempest Therapeutics, Olatec Therapeutics, A*STAR Singapore, Sanofi, Leap Therapeutics, GSK and AstraZeneca; personal fees from Merck & Co., Novartis, G1 Therapeutics and CE Concepts; travel fees from ASCO and ASCI and patents related to dendritic cell vaccines, immunotherapy biomarkers and methods for augmenting anti-PD-1 therapy. CC reports a pending patent related to high-affinity T-cell receptors that target the Merkel polyomavirus. JT reports consulting/advisory fees from Merck & Co, Bristol-Myers Squibb, AstraZeneca and Compugen; and a grant from Bristol-Myers Squibb. EJ, MK and BHM are employees of Merck & Co. SPF reports research funding from Merck. MAC reports research funding from Merck & Co. SLT reports that she or an immediate family member has stock and other ownership interests in Aduro Biotech, DNAtrix, Dracen Pharmaceuticals, Dragonfly Therapeutics, Ervaxx, Five Prime Therapeutics, Potenza Therapeutics, RAPT, Tizona Therapeutics, Trieza Therapeutics and WindMIL; a consulting or advisory role in Amgen, DNAtrix, Dragonfly Therapeutics, Dynavax, Ervaxx, Five Prime Therapeutics,

Immunocore, Immunomic Therapeutics, Janssen Pharmaceuticals, MedImmune/AstraZeneca, Merck & Co., RAPT and WindMIL; research grants from Bristol-Myers Squibb and Compugen; patents, royalties and/or other intellectual property with Aduro Biotech, Arbor Pharmaceuticals, Bristol-Myers Squibb, Immunomic Therapeutics, NexImmune and WindMIL and travel, accommodations, and expenses from Bristol-Myers Squibb, Dragonfly and Five Prime Therapeutics.

Patient consent for publication Not required.

Ethics approval This study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonisation Guidelines for Good Clinical Practice. All patients provided written informed consent before study entry. The protocol was approved by the Institutional Review Board at each participating center.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request.

ORCID iDs
Paul Nghiem http://orcid.org/0000-0003-2784-963X
Shailender Bhatia http://orcid.org/0000-0002-3816-2238
Evan J Lipson http://orcid.org/0000-0003-2976-0911
Tomoko Akaike http://orcid.org/0000-0003-4525-6408
Elad Sharon http://orcid.org/0000-0002-0044-9719

REFERENCES

1 Harms KL, Healy MA, Nghiem P, *et al*. Analysis of prognostic factors from 9387 merkel cell carcinoma cases forms the basis for the new 8th edition AJCC staging system. *Ann Surg Oncol* 2016;23:3564–71.
2 Paulson KG, Park SY, Vandeven NA, *et al*. Merkel cell carcinoma: Current US incidence and projected increases based on changing demographics. *J Am Acad Dermatol* 2018;78:457–63.
3 Engels EA, Frisch M, Goedert JJ, *et al*. Merkel cell carcinoma and HIV infection. *Lancet* 2002;359:497–8.
4 Clarke CA, Robbins HA, Tatalovich Z, *et al*. Risk of Merkel cell carcinoma after solid organ transplantation. *J Natl Cancer Inst* 2015;107:dju382.
5 Koljonen V, Kukko H, Pukkala E, *et al*. Chronic lymphocytic leukaemia patients have a high risk of Merkel-cell polyomavirus DNA-positive Merkel-cell carcinoma. *Br J Cancer* 2009;101:1444–7.
6 Heath M, Jaimes N, Lemos B, *et al*. Clinical characteristics of Merkel cell carcinoma at diagnosis in 195 patients: the AEIOU features. *J Am Acad Dermatol* 2008;58:375–81.
7 Lipson EJ, Vincent JG, Loyo M, *et al*. PD-L1 expression in the Merkel cell carcinoma microenvironment: association with inflammation, Merkel cell polyomavirus and overall survival. *Cancer Immunol Res* 2013;1:54–63.
8 Feng H, Shuda M, Chang Y, *et al*. Clonal integration of a polyomavirus in human Merkel cell carcinoma. *Science* 2008;319:1096–100.
9 Jing L, Ott M, Church CD, *et al*. Prevalent and Diverse Intratumoral Oncoprotein-Specific CD8$^+$ T Cells within Polyomavirus-Driven Merkel Cell Carcinomas. *Cancer Immunol Res* 2020;8:648–59.
10 Afanasiev OK, Yelistratova L, Miller N, *et al*. Merkel polyomavirus-specific T cells fluctuate with merkel cell carcinoma burden and express therapeutically targetable PD-1 and Tim-3 exhaustion markers. *Clin Cancer Res* 2013;19:5351–60.
11 Goh G, Walradt T, Markarov V, *et al*. Mutational landscape of MCPyV-positive and MCPyV-negative Merkel cell carcinomas with implications for immunotherapy. *Oncotarget* 2016;7:3403–15.
12 Harms PW, Vats P, Verhaegen ME, *et al*. The distinctive mutational spectra of polyomavirus-negative Merkel cell carcinoma. *Cancer Res* 2015;75:3720–7.
13 Wong SQ, Waldeck K, Vergara IA, *et al*. UV-associated mutations underlie the etiology of MCV-negative Merkel cell carcinomas. *Cancer Res* 2015;75:5228–34.
14 Knepper TC, Montesion M, Russell JS, *et al*. The genomic landscape of Merkel cell carcinoma and clinicogenomic biomarkers of response to immune checkpoint inhibitor therapy. *Clin Cancer Res* 2019;25:5961–71.
15 Iyer JG, Blom A, Doumani R, *et al*. Response rates and durability of chemotherapy among 62 patients with metastatic Merkel cell carcinoma. *Cancer Med* 2016;5:2294–301.
16 Nghiem P, Bhatia S, Lipson EJ, *et al*. Durable tumor regression and overall survival in patients with advanced Merkel cell carcinoma receiving pembrolizumab as first-line therapy. *J Clin Oncol* 2019;37:693–702.
17 D'Angelo SP, Bhatia S, Brohl AS, *et al*. Avelumab in patients with previously treated metastatic Merkel cell carcinoma: long-term data and biomarker analyses from the single-arm phase 2 javelin Merkel 200 trial. *J Immunother Cancer* 2020;8:e000674.
18 D'Angelo SP, Russell J, Lebbé C, *et al*. Efficacy and safety of first-line avelumab treatment in patients with stage IV metastatic Merkel cell carcinoma: a preplanned interim analysis of a clinical trial. *JAMA Oncol* 2018;4:e180077.
19 Merkel cell carcinoma. (Version 1.2020), 2019. Available: https://www.nccn.org/professionals/physician_gls/pdf/mcc.pdf [Accessed 01 Dec 2020].
20 Nghiem PT, Bhatia S, Lipson EJ, *et al*. PD-1 blockade with pembrolizumab in advanced Merkel-cell carcinoma. *N Engl J Med* 2016;374:2542–52.
21 Paulson KG, Carter JJ, Johnson LG, *et al*. Antibodies to Merkel cell polyomavirus T antigen oncoproteins reflect tumor burden in Merkel cell carcinoma patients. *Cancer Res* 2010;70:8388–97.
22 Moshiri AS, Doumani R, Yelistratova L, *et al*. Polyomavirus-negative Merkel cell carcinoma: a more aggressive subtype based on analysis of 282 cases using multimodal tumor virus detection. *J Invest Dermatol* 2017;137:819–27.
23 Edge S, Byrd DR, Compton CC. *Ajcc cancer staging Handbook*. 7th edn. New York: Springer, 2010.
24 Oken MM, Creech RH, Tormey DC, *et al*. Toxicity and response criteria of the eastern cooperative Oncology Group. *Am J Clin Oncol* 1982;5:649–56.
25 Topalian SL, Hodi FS, Brahmer JR, *et al*. Five-year survival and correlates among patients with advanced melanoma, renal cell carcinoma, or Non–Small cell lung cancer treated with nivolumab. *JAMA Oncology* 2019;5:1411–20.
26 Robert C, Long GV, Larkin J, *et al*. 1082MO 5-year characterization of complete responses in patients with advanced melanoma who received nivolumab plus ipilimumab (NIVO+IPI) or NIVO alone. *Annals of Oncology* 2020;31:S734–5.
27 Topalian SL, Sznol M, McDermott DF, *et al*. Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. *J Clin Oncol* 2014;32:1020–30.
28 Miller NJ, Bhatia S, Parvathaneni U, *et al*. Emerging and mechanism-based therapies for recurrent or metastatic Merkel cell carcinoma. *Curr Treat Options Oncol* 2013;14:249–63.
29 Waterhouse DM, Garon EB, Chandler J, *et al*. Continuous versus 1-year fixed-duration nivolumab in previously treated advanced non-small-cell lung cancer: CheckMate 153. *J Clin Oncol* 2020;38:3863–73.
30 Robert C, Ribas A, Hamid O, *et al*. Durable complete response after discontinuation of pembrolizumab in patients with metastatic melanoma. *J Clin Oncol* 2018;36:1668–74.
31 Garon EB, Hellmann MD, Rizvi NA, *et al*. Five-Year overall survival for patients with advanced Non–Small-Cell lung cancer treated with pembrolizumab: results from the phase I KEYNOTE-001 study. *J Clin Oncol* 2019;37:2518–27.
32 Templeton AJ, McNamara MG, Šeruga B, *et al*. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. *J Natl Cancer Inst* 2014;106:dju124.
33 Malietzis G, Giacometti M, Kennedy RH, *et al*. The emerging role of neutrophil to lymphocyte ratio in determining colorectal cancer treatment outcomes: a systematic review and meta-analysis. *Ann Surg Oncol* 2014;21:3938–46.
34 Zaragoza J, Kervarrec T, Touzé A, *et al*. A high neutrophil-to-lymphocyte ratio as a potential marker of mortality in patients with Merkel cell carcinoma: a retrospective study. *J Am Acad Dermatol* 2016;75:712–21.

35 Bartlett EK, Flynn JR, Panageas KS, *et al*. High neutrophil-to-lymphocyte ratio (NLR) is associated with treatment failure and death in patients who have melanoma treated with PD-1 inhibitor monotherapy. *Cancer* 2020;126:76–85.
36 Hasegawa T, Yanagitani N, Utsumi H, *et al*. Association of high neutrophil-to-lymphocyte ratio with poor outcomes of pembrolizumab therapy in high-PD-L1-expressing non-small cell lung cancer. *Anticancer Res* 2019;39:6851–7.
37 Lalani A-KA, Xie W, Martini DJ, *et al*. Change in neutrophil-to-lymphocyte ratio (NLR) in response to immune checkpoint blockade for metastatic renal cell carcinoma. *J Immunother Cancer* 2018;6:5.
38 Ameratunga M, Chénard-Poirier M, Moreno Candilejo I, *et al*. Neutrophil-Lymphocyte ratio kinetics in patients with advanced solid tumours on phase I trials of PD-1/PD-L1 inhibitors. *Eur J Cancer* 2018;89:56–63.
39 Ascierto ML, Makohon-Moore A, Lipson EJ, *et al*. Transcriptional mechanisms of resistance to anti-PD-1 therapy. *Clin Cancer Res* 2017;23:3168–80.
40 Kluger HM, Tawbi HA, Ascierto ML, *et al*. Defining tumor resistance to PD-1 pathway blockade: recommendations from the first meeting of the SITC immunotherapy resistance Taskforce. *J Immunother Cancer* 2020;8:e000398.
41 Ravi P, Mantia C, Su C, *et al*. Evaluation of the safety and efficacy of immunotherapy rechallenge in patients with renal cell carcinoma. *JAMA Oncol* 2020;6:1606.
42 Betof Warner A, Palmer JS, Shoushtari AN, *et al*. Long-term outcomes and responses to retreatment in patients with melanoma treated with PD-1 blockade. *J Clin Oncol* 2020;38:1655–63.
43 Sheth S, Gao C, Mueller N, *et al*. Durvalumab activity in previously treated patients who stopped durvalumab without disease progression. *J Immunother Cancer* 2020;8:e000650.
44 Robert C, Ribas A, Schachter J, *et al*. Pembrolizumab versus ipilimumab in advanced melanoma (KEYNOTE-006): post-hoc 5-year results from an open-label, multicentre, randomised, controlled, phase 3 study. *Lancet Oncol* 2019;20:1239–51.
45 LoPiccolo J, Schollenberger MD, Dakhil S, *et al*. Rescue therapy for patients with anti-PD-1-refractory Merkel cell carcinoma: a multicenter, retrospective case series. *J Immunother Cancer* 2019;7:170.
46 Bhatia S, Miller NJ, Lu H, *et al*. Intratumoral G100, a TLR4 agonist, induces antitumor immune responses and tumor regression in patients with Merkel cell carcinoma. *Clin Cancer Res* 2019;25:1185–95.
47 Song L, Bretz AC, Gravemeyer J, *et al*. The HDAC inhibitor Domatinostat promotes cell-cycle arrest, induces apoptosis, and increases immunogenicity of Merkel cell carcinoma cells. *J Invest Dermatol* 2020:32074–1.
48 Knackstedt R, Sussman TA, McCahon L, *et al*. Pre-treated anti-PD-1 refractory Merkel cell carcinoma successfully treated with the combination of PD-1/PD-L1 axis inhibitors and TVEC: a report of two cases. *Ann Oncol* 2019;30:1399–400.
49 Tabachnick-Cherny S, Pulliam T, Church C, *et al*. Polyomavirus-driven Merkel cell carcinoma: prospects for therapeutic vaccine development. *Mol Carcinog* 2020;59:807–21.
50 Topalian SL, Bhatia S, Amin A, *et al*. Neoadjuvant nivolumab for patients with resectable Merkel cell carcinoma in the CheckMate 358 trial. *J Clin Oncol* 2020;38:2476–87.

SUPPLEMENTAL MATERIAL

Three-year survival, correlates, and salvage therapies in patients receiving first line pembrolizumab for advanced Merkel cell carcinoma

TABLE OF CONTENTS

TABLE S1: DISPOSITION OF TREATMENT **2**

TABLE S2: ASSOCIATION OF OBJECTIVE RESPONSE (CR + PR) WITH BASELINE DEMOGRAPHICS, TUMOR AND TREATMENT CHARACTERISTICS **3**

TABLE S3: ADVERSE EVENTS **5**

TABLE S4: IMMUNE-MEDIATED ADVERSE EVENTS AND INFUSION REACTIONS **7**

TABLE S5: SUBSEQUENT TREATMENTS RECEIVED **8**

TABLE S6: SUBSEQUENT TREATMENTS RECEIVED FOR MCC PROGRESSION AFTER ON-STUDY PEMBROLIZUMAB, IN CHRONOLOGICAL ORDER ON A PER-PATIENT BASIS **10**

FIGURE S1: CORRELATION OF BASELINE TUMOR BURDEN WITH CLINICAL OUTCOMES **13**

FIGURE S2: CORRELATIONS OF PERIPHERAL BLOOD NEUTROPHIL-TO-LYMPHOCYTE RATIO (NLR) AND ABSOLUTE LYMPHOCYTE COUNT (ALC) WITH CLINICAL OUTCOMES **14**

Table S1: Disposition of treatment[a].

Nghiem P, *et al. J Immunother Cancer* 2021; 9:e002478. doi: 10.1136/jitc-2021-002478

Patient Disposition	**No. patients (N = 50)**	**%**
Completed treatment[b]	12	24
Discontinued treatment	37	74
Adverse event	13	26
Death	2	4
Physician decision	2	4
Progressive disease	19	38
Patient withdrawal	1	2
Status not recorded	1	2

[a]Each patient is counted once based on the latest corresponding disposition record.

[b]Patients who completed treatment received 2 years of pembrolizumab therapy.

Table S2: Association of objective response (CR + PR) with baseline demographics, tumor and treatment characteristics. Odds ratios with 95% CI are presented.

	Covariate	**CR/PR, n**	**SD/PD, n**	**OR**	**95% CI**
2 years of therapy Completed vs Discontinued	Discontinued	19	17	4.473	0.86-23.37
	Completed	10	2		
PD-L1 expression in tumor[a] Positive vs Negative	<1%	13	10	1.442	0.44-4.74
	≥1%	15	8		
Age ≥70 vs < 70 years	<70 years	13	10	1.231	0.39-3.86
	≥70 years	16	10		
Viral Status Positive vs Negative	Negative	10	8	1.267	0.39-4.11
	Positive	19	12		
Sex Female vs Male	Male	19	14	1.228	0.36-4.18
	Female	10	6		
Visceral and/or bone site Yes vs no	No	12	10	1.204	0.36-3.97
	Yes	13	9		
Skin, subcutaneous, and/or lymph node site Yes vs no	No	6	2	0.407	0.07-2.27
	Yes	22	18		
Tumor size ≥median vs <median	Median <56 mm	13	9	0.969	0.30-3.14
	Median ≥56	14	10		

	mm				
ECOG PS 1 vs 0	0	16	8	0.542	0.17- 1.72
	1	13	12		

CI, confidence interval; CR, complete response; ECOG PS, Eastern Cooperative Oncology Group Performance Status; OR, odds ratio; PD, progressive disease; PD-L1 programmed death ligand 1; PR, partial response; SD, stable disease.

[a]A tumor specimen was scored as being PD-L1 positive if ≥1% of tumor cells expressed cell surface PD-L1, assessed by immunohistochemistry.

Table S3: Adverse events

	No. patients **N = 50**	**%**
Patients with one or more AEs	50	100
with TRAEs[a]	49	98
with toxicity grade 3-5 AEs	29	58
with toxicity grade 3-5 TRAEs	15	30
with serious AEs	22	44
with serious TRAEs	11	22
who died	1	2
who died due to a TRAE	1	2
discontinued drug due to an AE	11	22
discontinued drug due to a TRAE	8	16
discontinued drug due to a serious AE	4	8
discontinued drug due to a serious TRAE	3	6

AE, adverse event; TRAE, treatment-related adverse event.

[a]Determined by the investigator to be related to the drug.

Non-serious adverse events up to 30 days after last dose and serious adverse events up to 90 days after last dose are included.

MedDRA V20.1 preferred terms “neoplasm progression”, “malignant neoplasm progression” and “disease progression” not related to the drug are excluded.

AEs were graded according to NCI CTCAE, Version 4.0.

Table S4: Immune-mediated adverse events and infusion reactions[a].

	No. patients **N = 50**	**%**
Patients with one or more AEs	17	34
with TRAEs[b]	16	32
with toxicity grade 3-5 AEs	7	14
with toxicity grade 3-5 TRAEs	6	12
with serious AEs	6	12
with serious TRAEs	5	10
who died	0	0
discontinued drug due to an AE	7	14
discontinued drug due to a TRAE	6	12
discontinued drug due to a serious AE	4	8
discontinued drug due to a serious TRAE	3	6

AE, adverse event; TRAE, treatment-related adverse event.

[a]Immune-mediated adverse events were based on a list of terms specified by the sponsor and were included regardless of treatment attribution by investigators.

[b]Determined by the investigator to be related to the drug.

Table S5: Subsequent treatments received.

	No. patients **N = 50**	**%**
Patients with one or more subsequent oncologic therapies[a]	22	44
Chemotherapy	7	14
Carboplatin	5	10
Carboplatin + etoposide	1	2
Cyclophosphamide	1	2
Doxorubicin	1	2
Etoposide	5	10
Topotecan	2	4
Vincristine	1	2
Immunotherapy	17	34
Avelumab	3	6
Glucopyranosyl lipid A	1	2
Immunotherapy (unspecified)	1	2
Ipilimumab	4	8
Natural killer cells	2	4

Nivolumab	4	8
Pembrolizumab	10	20
Talimogene laherparepvec	1	2
Other	8	16
Octreotide acetate	3	6
Radiation therapy	7	14

Each patient is counted only once for each applicable row and column.

[a]Five patients started new cancer therapies without documented disease progression.

Table S6: Subsequent treatments received for MCC progression after on-study pembrolizumab, in chronological order on a per-patient basis.

	Patient Number[a]	**Subsequent Therapies (in order initiated)**				
		1st	**2nd**	**3rd**	**4th**	**5th**
Patients with CR, PR, or SD whose disease relapsed	1	Carboplatin + etoposide	Ipilimumab + pembrolizumab			
	2	Ipilimumab + nivolumab	Pembrolizumab			
	3	Pembrolizumab				
	4	Octreotide acetate	Radiation therapy	Avelumab	Radiation therapy	
	5	Pembrolizumab	Ipilimumab + nivolumab	Carboplatin + etoposide		
	6	Topotecan				
	7	Octreotide acetate				
	8	Radiation therapy	Nivolumab			
	9[a]	Pembrolizumab				

	10	Ipilimumab				
	11	None				
Patients with initial PD	12[a]	Pembrolizumab				
	13[a]	None				
	14	None				
	15	Carboplatin + etoposide	Natural killer cells	Carboplatin + etoposide	Radiation therapy	
	16	Glucopyranosyl lipid A	Radiation therapy	Octreotide acetate	Natural killer cells	Pembrolizumab
	17	Avelumab + radiation therapy	Radiation therapy	Avelumab	Immunotherapy (unspecified)	
	18	None				
	19	Carboplatin + etoposide				
	20	None				
	21	None				

	22	None				
	23	Carboplatin + etoposide	Avelumab	Topotecan	Cyclophosphamide + doxorubicin + vincristine	
	24	Carboplatin + etoposide				
	25	None				
	26	None				
	27	None				

CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.

[a]Patient ID numbers refer to patients as shown in Figures 2C and 2D.

[b]Patients 9, 12, and 13 received standard of care radiation therapy to control symptomatic metastases while receiving pembrolizumab on study, as allowed per the study protocol.

Figure S1: Correlation of baseline tumor burden with clinical outcomes. **(A)** Association of baseline tumor burden with survival at 30 months in 47 evaluable patients. Baseline tumor dimensions were not available for 3 patients; 1 was alive and 2 had died at 30 months. **(B)** Association of baseline tumor burden with best overall response in 46 evaluable patients. Baseline tumor dimensions were not available for 3 patients, and response data were not available for 1 additional patient. CR/PR, complete or partial response. SD/PD, stable or progressive disease.

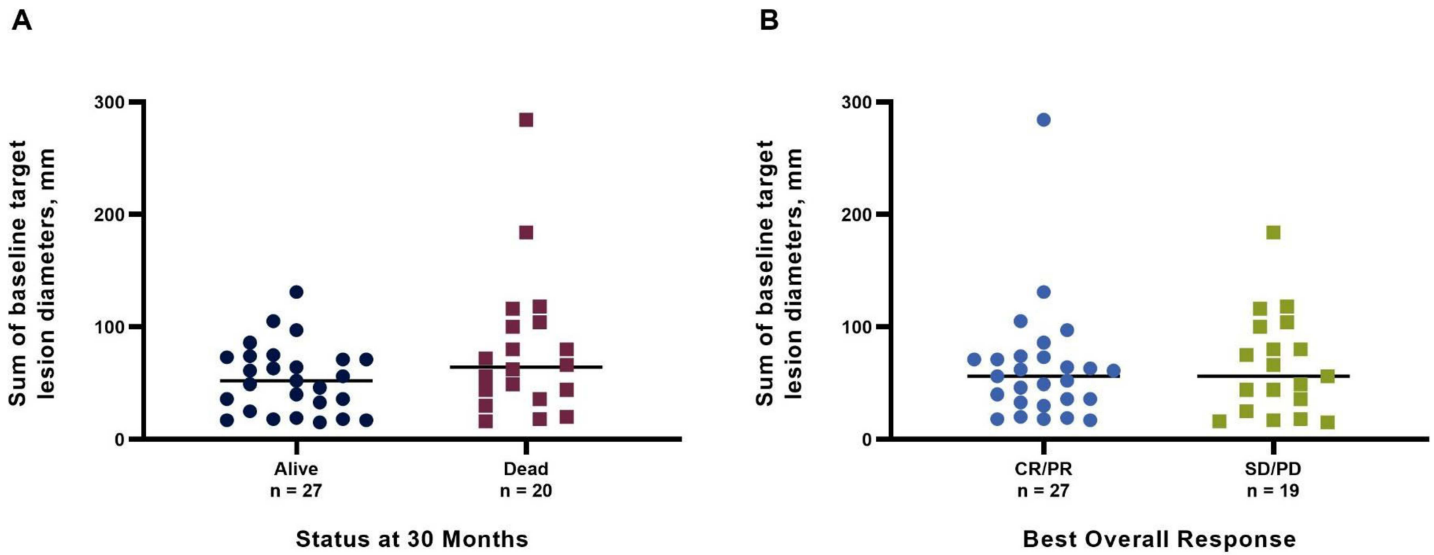
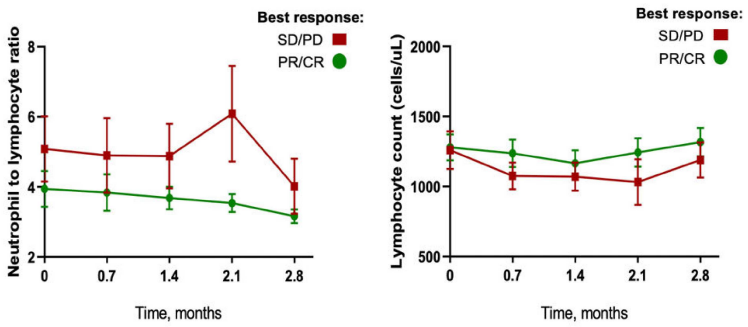


Figure S2: Correlations of peripheral blood neutrophil-to-lymphocyte ratio (NLR) and absolute lymphocyte count (ALC) with clinical outcomes. NLR was calculated as the ratio of the absolute neutrophil and lymphocyte counts (cells/uL). Mean values and SEM are shown. **(A)** NLR and ALC in patients with objective response (complete or partial response [CR/PR]) versus no response (stable or progressive disease [SD/PD]). Statistical comparisons for individual time points used t-test allowing for unequal variance. The p-value for analysis across all time points is based on mixed model, with treatment interval and response status (PR/CR vs. SD/PD) as fixed effects, with random intercept. **(B)** NLR and ALC in patients with 30 months' survival follow-up after study treatment initiation. Statistical comparisons for individual time points used t-test allowing for unequal variance. The p-value for analysis across all time points is based on mixed model, with treatment interval and 30-month survival status as fixed effects, with random intercept.

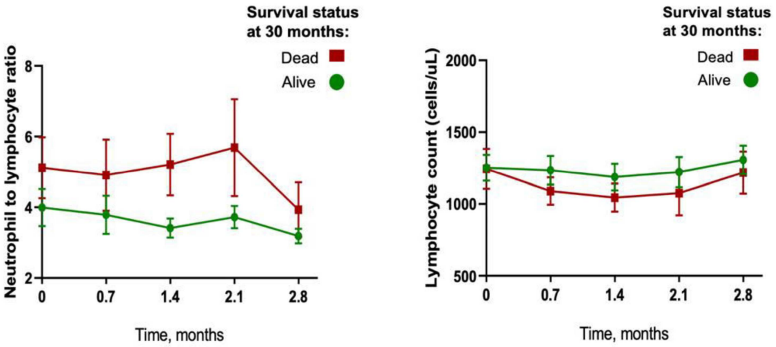
A



NLR	PR/CR		SD/PD		
Time, months	Mean	N	Mean	N	p-value
0	3.943	n = 27	5.084	n = 18	0.292
0.7	3.838	n = 27	4.896	n = 17	0.383
1.4	3.683	n = 26	4.878	n = 16	0.234
2.1	3.539	n = 24	6.085	n = 11	0.095
2.8	3.160	n = 24	4.017	n = 8	0.322
Mixed Model					**0.0427**

ALC	PR/CR		SD/PD		
Time, months	Mean	N	Mean	N	p-value
0	1279.3	n = 27	1258.8	n = 18	0.9007
0.7	1237.4	n = 27	1075.6	n = 17	0.2444
1.4	1165.5	n = 26	1070.5	n = 16	0.4901
2.1	1242.6	n = 24	1031.9	n = 11	0.2864
2.8	1317.0	n = 24	1189.1	n = 8	0.4393
Mixed Model					**0.2995**

B



NLR	Alive (30mo FU)		(30mo FU)		
Time, months	Mean	N	Mean	N	p-value
0	3.9904	n = 26	5.1195	n = 20	0.2471
0.7	3.7877	n = 26	4.9095	n = 18	0.3355
1.4	3.4123	n = 25	5.2055	n = 17	0.0644
2.1	3.7126	n = 24	5.6879	n = 11	0.1895
2.8	3.1887	n = 24	3.9326	n = 8	0.1966
Mixed Model					**0.0282**

ALC	Alive (30mo FU)		(30mo FU)		
Time, months	Mean	N	Mean	N	p-value
0	1251.9	n = 26	1244.2	n = 20	0.9621
0.7	1234.5	n = 26	1088.8	n = 18	0.2984
1.4	1187.4	n = 25	1043.9	n = 17	0.2948
2.1	1222.3	n = 24	1076.1	n = 11	0.446
2.8	1307.1	n = 24	1218.6	n = 8	0.6269
Mixed Model					**0.2501**