Research Article

Cancer Immunology Research

Liver Metastasis and Treatment Outcome with Anti-PD-1 Monoclonal Antibody in Patients with Melanoma and NSCLC

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Abstract

We explored the association between liver metastases, tumor $CD8^+$ T-cell count, and response in patients with melanoma or lung cancer treated with the anti-PD-1 antibody, pembrolizumab. The melanoma discovery cohort was drawn from the phase I Keynote 001 trial, whereas the melanoma validation cohort was drawn from Keynote 002, 006, and EAP trials and the non–small cell lung cancer (NSCLC) cohort from Keynote 001. Liver metastasis was associated with reduced response and shortened progression-free survival [PFS; objective response rate (ORR), 30.6%; median PFS, 5.1 months] compared with patients without liver metastasis (ORR, 56.3%; median PFS, 20.1 months) $P \leq 0.0001$, and confirmed in the validation cohort ($P = 0.0006$). The presence of liver metastasis significantly increased the likelihood of progression (OR, 1.852; $P < 0.0001$). In a subset of biopsied patients ($n = 62$), liver metastasis was associated with reduced $CD8^+$ T-cell density at the invasive tumor margin (liver metastasis$^+$ group, $n = 547 \pm 164.8$; liver metastasis$^-$ group, $n = 1{,}441 \pm 250.7$; $P < 0.016$). A reduced response rate and shortened PFS was also observed in NSCLC patients with liver metastasis [median PFS, 1.8 months; 95% confidence interval (CI), 1.4–2.0], compared with those without liver metastasis ($n = 119$, median PFS, 4.0 months; 95% CI, 2.1–5.1), $P = 0.0094$. Thus, liver metastatic patients with melanoma or NSCLC that had been treated with pembrolizumab were associated with reduced responses and PFS, and liver metastases were associated with reduced marginal $CD8^+$ T-cell infiltration, providing a potential mechanism for this outcome. *Cancer Immunol Res; 5(5); 417–24. ©2017 AACR.*

Introduction

Antibodies that block binding between programmed death 1 (PD-1) and its ligands, PD-L1 or PD-L2, have shown marked clinical activity in many malignancies, including metastatic melanoma (1–7), non–small cell lung cancer (NSCLC; refs. 8–11), and other cancers (12). The diversity of different cancers in which PD-1/PD-L1–directed therapies have shown efficacy has emphasized that the biological importance of PD-1 on activated, tumor-associated T cells (13–15) transcends histologic subtype. However, specific inter- and even intrapatient features define the distinct nature of a given tumor's immune microenvironment that can modulate the likelihood of benefit from PD-1/PD-L1 blockade.

The presence of a T-cell infiltrate and PD-L1 expression on tumor and tumor stroma represents a stratification factor that has shown predictive value in various cancer types (4, 16, 17). It has been noted, though, that PD-L1 expression is only modestly predictive of response. Tumor $CD8^+$ T-cell infiltration at the invasive margin has been shown to be predictive of response in melanoma (18). Less attention has been paid to the clinical variables that may impact responsiveness to PD-1/PD-L1 blockade and may provide insight into characteristics of both host and tumor that ultimately shape the tumor microenvironment. One criticism of efforts in predictive modeling for immunotherapy focused on single-assay biomarkers, such

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as PD-L1 expression, has been the lack of integration of clinical variables into the models and consequently the reduced usefulness of these models (19).

Recent reports have suggested an association between the presence of lung metastases and clinical benefit with pembrolizumab (3). Conversely, although not contradictorily, our group previously noted that the presence of liver metastases was associated with poor prognosis in an initial subset of melanoma patients receiving pembrolizumab (20). In this study, we sought to determine the relationship between metastatic pattern, organ-specific differential T-cell infiltration, and treatment outcome in patients treated with pembrolizumab.

Materials and Methods

Study design

Between December 2011 and October 2013, 223 patients with melanoma were treated with pembrolizumab at University of California, San Francisco (UCSF, San Francisco, CA), University of California, Los Angeles (UCLA; Los Angeles, CA), or the Angeles Clinic as part of KEYNOTE-001 (ClinicalTrials.gov NCT01295827). This trial was a large phase I clinical trial that examined the safety and efficacy of pembrolizumab in patients with multiple solid tumor malignancies. An independent validation cohort was comprised of 113 patients treated with pembrolizumab between February 2013 and September 2015 at UCSF or at the University Hospital of Zürich (Zürich, Switzerland) on Keynotes 002 and 006, and Merck EAP (ClinicalTrials.gov identifiers, P002: NCT 01704287, P006: NCT01866319, EAP: NCT02083484). A comparison cohort of 165 patients with advanced non–small cell lung cancer treated with pembrolizumab at UCSF, MSKCC, or UCLA as part of KEYNOTE-001 (ClinicalTrials.gov NCT01295827) was also examined.

In the discovery and validation melanoma cohorts, as well as the NSCLC comparison cohort, pembrolizumab was administered intravenously at 2 or 10 mg/kg every 2 or 3 weeks as described previously (3). Efficacy was determined in the melanoma cohorts by RECIST v 1.1 using CT imaging at 12 and 16 weeks after the first infusion, and every 12 weeks thereafter (21). Progression-free survival (PFS) was calculated from the date of randomization to the date of progression or death. In the NSCLC cohort, efficacy was determined using immune-related response criteria, and scans were repeated every 9 weeks (10).

Available efficacy and immunologic data as of July 1, 2015, were included in all the analyses. The efficacy analysis included two endpoints: (i) best overall response was defined as the best tumor response from the start of treatment to the time of disease progression or death; and (ii) PFS was defined as the interval between the date of enrollment and the date of progression or death (or the last date of clinic visit where the patient was known not to have had radiological or clinical progression). Best overall response was determined from investigator-reported data according to RECIST 1.1 criteria.

Tumor sample procurement

Melanoma patients underwent an optional biopsy before starting treatment. Of these, 61 samples were available for IHC analysis. Biopsy collection and analyses were approved by IRBs 11-003066 (UCLA) and 13-12246 (UCSF).

Table 1. Baseline demographic and clinical characteristics of patients with and without liver metastases in discovery and validation cohort populations

	Discovery ($n = 223$)		Validation ($n = 113$)	
Characteristics	Liver − ($n = 151$)	Liver + ($n = 72$)	Liver − ($n = 77$)	Liver + ($n = 36$)
Median age – y (range)	64 (26–95)	65 (19–77)	61 (21–91)	61.5 (28–87)
Sex – *n* (%)				
Female	53 (35%)	25 (35%)	27 (35%)	13 (36%)
Male	98 (65%)	47 (65%)	50 (65%)	23 (64%)
ECOG performance status – *n* (%)				
0	116 (77%)	49 (68%)	63 (82%)	20 (56%)
1	35 (23%)	23 (32%)	14 (18%)	16 (44%)
LDH level – *n* (%)				
Elevated (>199)	58 (38%)	43 (60%)	49 (64%)	27 (75%)
Normal (≤199)	93 (62%)	29 (40%)	28 (36%)	9 (25%)
Primary site of melanoma – *n* (%)				
Cutaneous	125 (83%)	47 (65.3%)	66 (85.7%)	30 (83.3%)
Mucosal	14 (9%)	5 (6.9%)	2 (2.6%)	3 (8. 3%)
Unknown	10 (7%)	1 (1.4%)	9 (11.7%)	3 (8. 3%)
Uveal	2 (1%)	19 (26.4%)	0 (0%)	0 (0%)
Previous targeted therapy – *n* (%)				
No	119 (79%)	57 (79%)	57 (74%)	27 (75%)
Yes	32 (21%)	15 (21%)	20 (26%)	9 (25%)
Previous ipilimumab – *n* (%)				
No	83 (55%)	37 (51%)	12 (16%)	6 (17%)
Yes	68 (45%)	35 (49%)	65 (84%)	30 (83%)
BRAF V600E status – *n* (%)				
Mutated	41 (27%)	15 (21%)	25 (32%)	8 (22%)
Wild type	110 (73%)	57 (79%)	52 (68%)	28 (78%)
Brain metastasis – *n* (%)				
No	119 (79%)	65 (90%)	59 (77%)	27 (75%)
Yes	32 (21%)	7 (10%)	18 (23%)	9 (25%)
Lung metastasis – *n* (%)				
No	73 (48%)	42 (58%)	27 (35%)	15 (42%)
Yes	78 (52%)	30 (42%)	50 (65%)	21 (58%)

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

IHC staining

Slides were stained with hematoxylin and eosin, S100, CD8, PD-1, and PD-L1 at the UCLA Clinical IHC Laboratory as described previously (18). All stained slides were evaluated in a blinded fashion by one dermatopathologist and one investigator trained to identify the features of melanoma. S100, an established melanoma marker, was used to define the histologic tumor margin. Slides were examined for the presence of CD8, PD-1, and PD-L1 at the invasive tumor margin as described previously (18).

Digital image acquisition and analysis

All slides were scanned at an absolute magnification of ×200 (resolution of 0.5 μm/pixel). An algorithm was designed on the basis of pattern recognition that quantified immune cells. Image analysis based on RGB (red, green, blue) spectra was used to detect all cells by counterstaining with hematoxylin (blue), and DAB or fast red. The imaging analysis algorithm calculated the density of CD8, PD-1, and PD-L1–positive cells (cells/mm^2).

Statistical analyses

Demographic and clinical characteristics were compared using the Kruskal–Wallis or Student *t* test for age and continuous variables, and χ^2 or Fisher exact test for categorical variables. Ninety-five percent confidence intervals (CI) were computed using the Wald confidence limits for the binomial proportion. Proportional hazard Cox regression was used to determine the

association of demographic and clinical variables with response and PFS. The full model included terms for metastatic location, age, gender, previous targeted therapy, BRAF status, baseline tumor burden, lactate dehydrogenase (LDH) level, and primary site of melanoma. PFS curves were constructed with the Kaplan–Meier method separately stratified by primary site of melanoma and metastatic location. Analyses were performed using SAS V9.4 (SAS Institute Inc.), SPSS V22 (IBM Corp.), and GraphPad Prism v6 (GraphPad Software, Inc.). All tests were two-sided with P values <0.05 considered statistically significant.

Results

Baseline clinical characteristics according to metastatic pattern

Table 1 shows the baseline characteristics of the melanoma patients in the discovery and validation cohorts stratified by metastatic pattern. Variables that were significantly associated with best overall response included gender, LDH concentration, prior ipilimumab therapy, and metastatic site (Supplementary Fig. S1). Pembrolizumab dosing and schedule were not included in the analysis, because multiple reports have independently examined this issue and confirmed the lack of association (3, 7, 22–24).

Multivariate analysis of prognostic factors including metastatic site

On the basis of the univariate analysis described above, we constructed a multivariate model including the entire melanoma population (Fig. 1). In the multivariate analysis, female gender, elevated LDH, ECOG > 0, and the presence of liver metastasis were all significantly correlated with worse PFS (Fig. 1). Other significant variables in multivariable analysis included prior ipilimumab treatment, whereas the history of brain metastasis, BRAF status, and prior targeted therapy were not significant.

PFS and objective response rate, by metastatic pattern

Having identified the significance of liver metastasis in the multivariate analysis of the entire melanoma population, we examined the relationship between liver metastasis and PFS

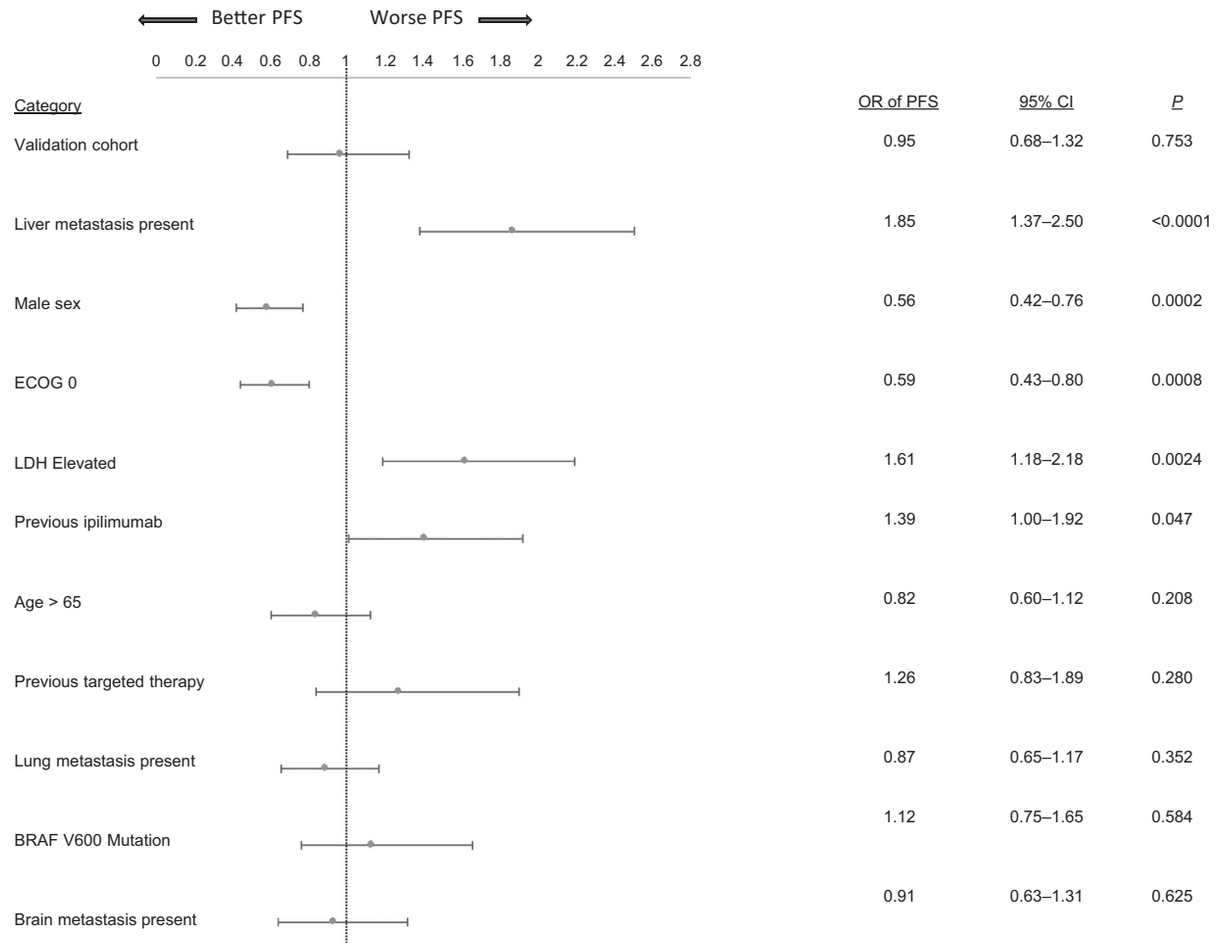

Figure 1.

Multivariable analysis of pretreatment prognostic factors. ORs were calculated for PFS in each subgroup. The statistical significance and the CIs are depicted on the right columns. The dotted vertical line designates the PFS for the entire cohort.

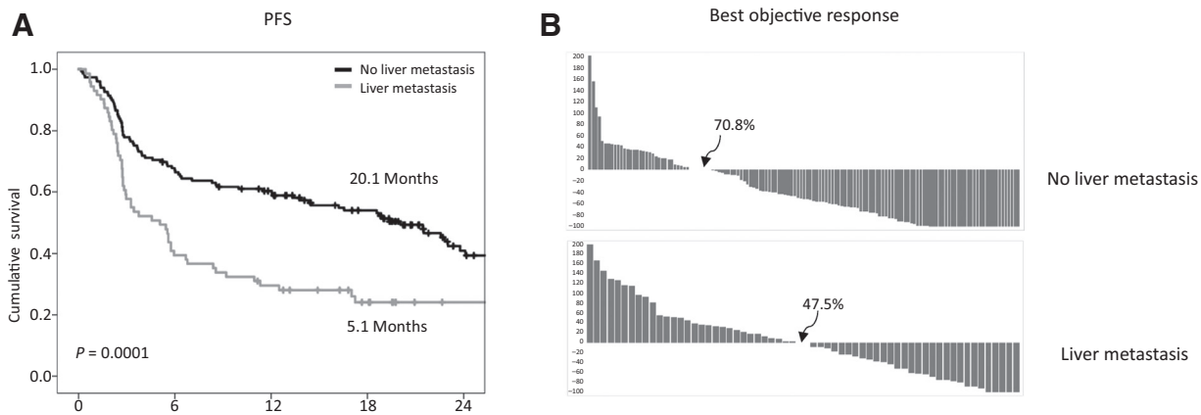

Figure 2.

PFS and response rate in patients with melanoma treated with pembrolizumab. **A** and **B,** The discovery cohort ($n = 223$). **A,** Kaplan–Meier PFS curve; patients with liver metastasis are shown in gray, whereas those without liver metastasis are shown in black. **B,** Waterfall plots for patients without and with liver metastasis.

and objective response rate (ORR) in the discovery cohort. In the discovery cohort (Fig. 2A and B), the outcome for the liver metastases groups was worse than the outcome of patients without liver involvement. For example, the median PFS was 5.1 months for patients with liver metastasis, whereas it was 20.1 months for patients without liver metastasis. This difference was statistically significant ($P < 0.0001$). This effect was confirmed in the validation cohort (Fig. 3A and B); patients with liver metastasis had a median PFS of 2.7 months versus a median PFS of 18.5 months for patients without liver involvement. This difference was statistically significant in the validation cohort as well ($P = 0.0006$).

Tumor margin CD8$^+$ T-cell count and response to pembrolizumab, by metastatic pattern

Having identified the importance of metastatic pattern to response, we investigated the relationship between the presence of preexisting tumor-associated T-cell infiltrates and metastatic pattern with quantitative IHC analysis of CD8, PD-1, and PD-L1 expression at the invasive margin in samples obtained from 61 patients before treatment (Fig. 4). Fewer CD8$^+$ T cells were found in the nonresponder group when compared with the responder group (responder group $n = 25$, nonresponder group $n = 35$, $P < 0.0001$, Fig. 4A). The CD8$^+$ T-cell count at the invasive margin was also significantly lower in the liver metastasis$^+$ group versus the liver metastasis$^-$ group (liver metastases group $n = 22$, mean count 547 ± 164.8; no liver metastases group, $n = 40$, mean count 1441 ± 250.7; $P < 0.016$; Fig. 4B). In the same tumor samples, PD-1 and PD-L1 expression by IHC was not significantly different in the liver metastases$^+$ cohort as compared with the liver metastases$^-$ cohort (Fig. 4C and D). Figure 4E shows examples of CD8, PD-1, and PD-L1 expression in samples obtained from distant metastatic tumors in terms of the presence or absence of liver metastases and response to pembrolizumab.

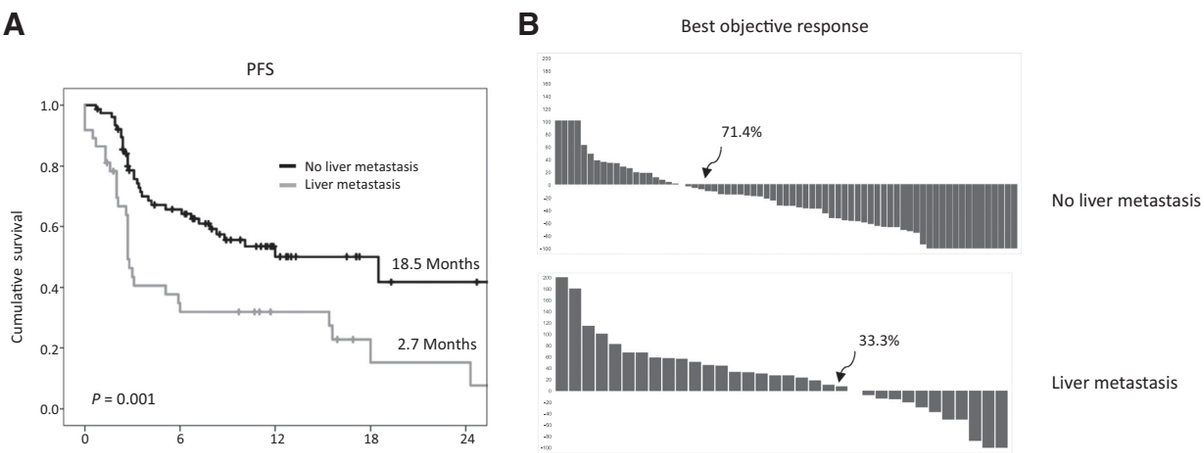

Figure 3.

PFS and response rate in the validation cohort ($n = 113$) patients with melanoma treated with pembrolizumab. **A,** Kaplan–Meier PFS curve with liver metastasis (gray) and those without liver metastasis (black). **B,** Waterfall plot for patients in the validation cohort. PFS was calculated from the time of randomization. Log-rank analysis was used to calculate the differences between these groups.

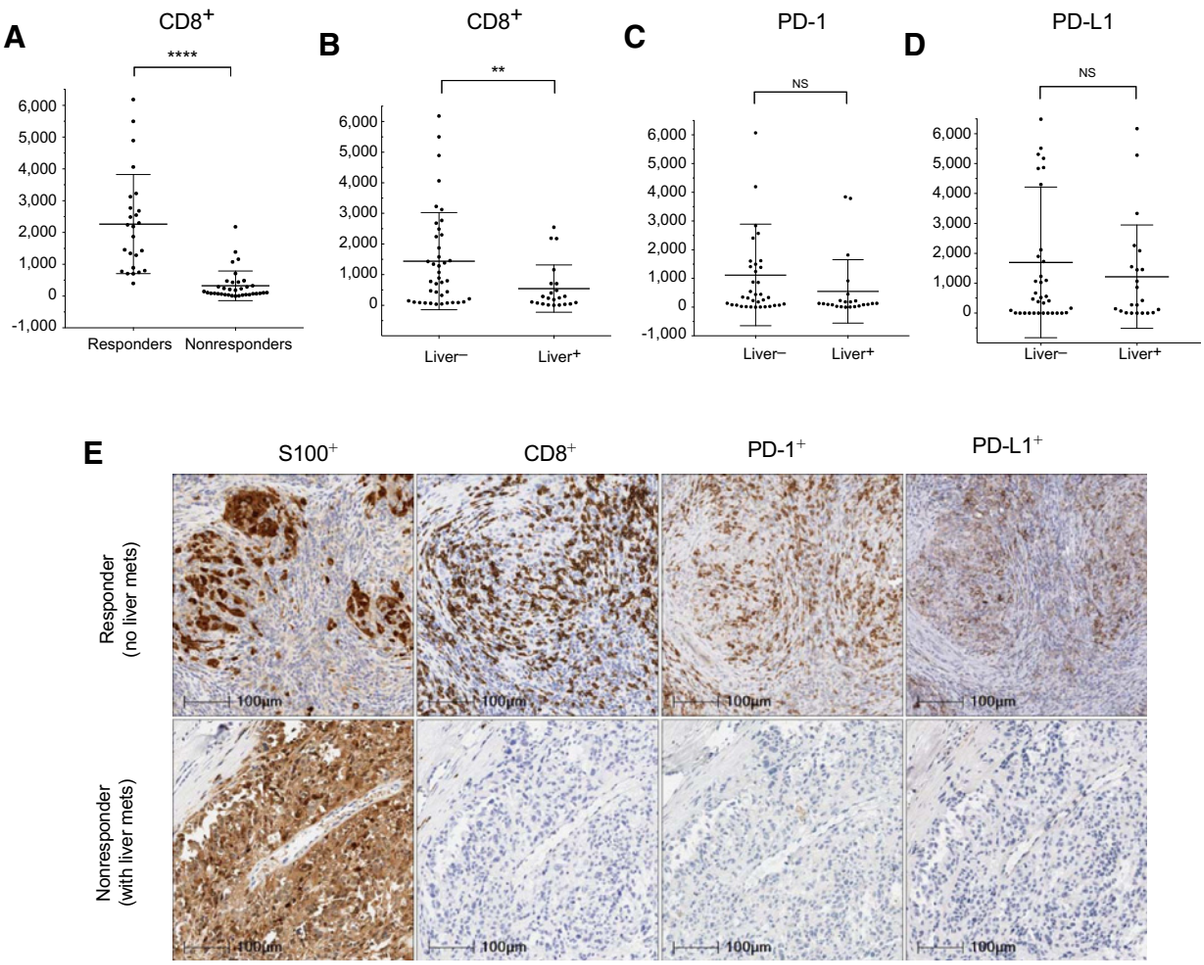

Figure 4.

IHC analysis of CD8, PD-1, and PD-L1 in samples obtained from patients before pembrolizumab, according to response and metastatic pattern. **A,** $CD8^+$ T-cell density by response category (responder group $n = 25$, nonresponder group $n = 35$; $P < 0.0001$). **B,** $CD8^+$ T-cell density by liver metastasis status (liver $metastasis^+$ group $n = 22$, mean count 547 ± 164.8; liver $metastases^-$ group $n = 40$, mean count 1,441 ± 250.7; $P < 0.016$). **C** and **D,** PD-1 and PD-L1, respectively, expression by liver metastasis. NS, not significant. **E,** Examples of CD8, PD-1, and PD-L1 expression in samples obtained from distant tumors in terms of metastatic location and response. Magnification, ×20. Serial cut tissue sections (2 μm) were stained for S100, CD8, PD-1, and PD-L1. **, <0.016; ****, <0.0001.

Patients with liver metastases also had significantly lower densities of $CD8^+$ T cells in distant nonliver metastases. We obtained archived tumor samples that represented 35 patients with confirmed melanoma metastases in the liver and in nonliver sites, but were never treated with pembrolizumab (Supplementary Fig. S2). We analyzed the presence of $CD8^+$ T cells in the nonliver biopsies from these patients. CD8 expression in the nonliver biopsies of the distant metastases group was comparable with the liver $metastases^+$ group (liver $metastases^+$ group, 546.9 ± 164.8; distant metastases of liver $metastases^+$ group, 479.1 ± 98.49, $P = 0.7079$) and significantly lower when compared with the liver $metastases^-$ group (liver $metastases^-$ group, 1,441 ± 250.7; $P \leq 0.0001$; distant metastases group, $P = 35$; liver $metastases^-$ group, $n = 40$, liver $metastases^+$ group, $n = 22$).

PFS and metastatic pattern in NSCLC

Given the association between liver metastasis and the outcome of pembrolizumab treatment in melanoma, we investigated whether this relationship could be seen in NSCLC. As with melanoma, there is a significant body of data on patients treated with pembrolizumab. In this tumor type, the PFS was also significantly reduced in patients with liver metastasis ($n = 46$, median PFS 1.82 months; 95% CI, 1.36–2.02) compared with those without liver metastasis ($n = 119$, median PFS 4.03 months; 95% CI, 2.12–5.09), $P = 0.0094$ (Kaplan–Meier curves for PFS, Fig. 5A). ORRs were also lower in the liver $metastases^+$ population compared with the liver $metastases^-$ population (waterfall plots, Fig. 5B).

Discussion

In this report, we investigated the clinical characteristics of nonresponders to pembrolizumab. In melanoma patients, we discovered that liver metastasis was independently predictive of reduced response and poor outcome. In a separate validation cohort, this relationship was confirmed. This effect is not due to

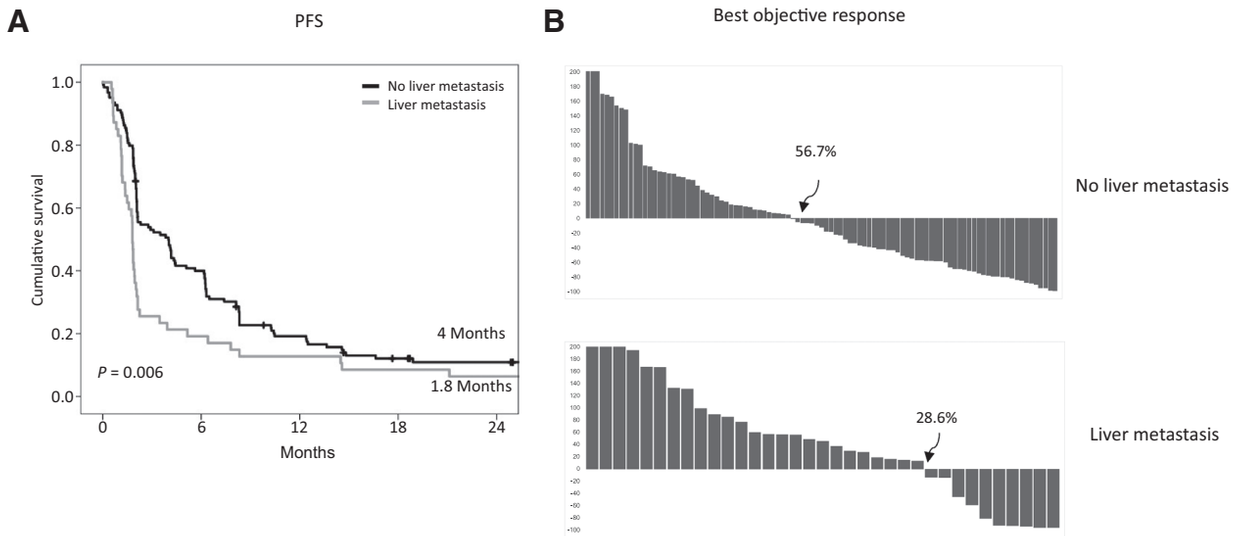

Figure 5.

PFS and response rate in patients with NSCLC treated with pembrolizumab. **A,** Kaplan–Meier PFS curve; patients with liver metastasis are shown in gray, whereas those without liver metastasis are shown in black. **B,** Waterfall plots for patients without and with liver metastasis.

advanced stage (M1C) alone (ORR for M1C, 45.5%) or due to the site of origin of melanoma (uveal melanoma patients were excluded from the validation cohort). Additional factors noted to be significantly associated with adverse outcome in the multivariable analysis included female gender, elevated LDH, and prior ipilimumab treatment.

The presence of liver metastases was associated with fewer infiltrating $CD8^+$ T cells at the invasive margin in distant tumors, a cellular signature that correlates with response to PD-1. We extended this observation in a set of patients who had cutaneous metastasis as well as liver metastasis. In this set of tumors, the cutaneous metastases also had depleted marginating $CD8^+$ T cells, suggesting that the effect of liver metastasis was systemic.

In a comparison cohort of patients with NSCLC, the presence of liver metastasis was associated with decreased likelihood of response to pembrolizumab. Although pembrolizumab has less activity in terms of ORR and PFS in NSCLC compared with melanoma (10, 11), the same pattern of reduced efficacy was seen in patients with liver metastasis.

The decreased probability of response to pembrolizumab seen in patients with liver metastasis can have several explanations. Liver-induced peripheral tolerance represents a well-established but poorly understood phenomenon that was initially described in the setting of orthotopic liver transplantation. Unlike heart or kidney allografts, liver allografts are accepted spontaneously in mice, rats, pigs, and even in humans, often without the need for histocompatibility or even in some instances, immunosuppression (25–27). In addition, liver allografts confer on the recipient tolerance to other transplanted organs from the same donor, suggesting that the transplanted liver can induce systemic immune tolerance (26, 28). Multiple mechanisms have been put forward to explain liver-induced systemic tolerance, including incomplete activation of $CD8^+$ T cells (29–32), trapping and deletion of activated $CD8^+$ cells (33, 34), poor $CD4^+$ T-cell activation (35), and Kupffer cells promoting activation of regulatory T cells (30). In addition, it appears that viral pathogens, in particular hepatitis C virus (HCV) and lymphocytic choriomeningitis virus, may exploit mechanisms of liver tolerance to evade antiviral CD8 responses, including the direct upregulation of PD-L1 on myeloid-derived Kupffer cells by HCV (36). Mechanistic studies using animal models may help to distinguish between these possibilities in the future. It is also possible that other unexamined variables may explain the findings we describe. Other studies have shown that baseline tumor size (37), tumor aneuploidy (38), tumor mutation burden (39), intestinal microbial flora (40), and tumor *wnt* pathway signaling (41) can all affect response to checkpoint inhibitors. It is certainly possible that these could be confounding factors in terms of response. Although these mechanistic studies and additional confirmatory studies are ongoing, the presence of liver metastasis should not be used to exclude patients from PD-1 therapy. Indeed, the response rate even in this group of patients exceeds the response rate reported for other therapies.

Disclosure of Potential Conflicts of Interest

M.D. Hellmann reports receiving other commercial research support from BMS and Genentech and is a consultant/advisory board member for AstraZeneca, BMS, Genentech, Janssen, Merck, and Novartis. O. Hamid has received speakers bureau honoraria from Amgen, BMS, Genentech, and Novartis, is a consultant/advisory board member for Amgen, BMS, Merck, Novartis, and Roche. M.A. Gubens is a consultant/advisory board member for BMS. J.M. Taube reports receiving a commercial research grant from Bristol-Myers Squibb and is a consultant/advisory board member for Astra Zeneca, Bristol-Myers Squibb, and Merck. E.J. Taylor is the chief executive officer at Naked Biome. B. Chmielowski has received speakers bureau honoraria from Genentech and Janssen, is a consultant/advisory board member for Amgen, Astellas, BMS, Eisai, Genentech, Immunocore, Lilly, and Merck. R. Dummer is a consultant/advisory board member for BMS and MSD. S.M. Goldinger is a consultant/advisory board member for BMS, MSD, Novaertis, and Roche. No potential conflicts of interest were disclosed by the other authors.

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and sponsors had no role in the design and conduct of the study, collection, management, analysis, and interpretation of the data, preparation, review, or approval of the manuscript, and decision to submit the manuscript for publication.

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