

Safety, Clinical Activity, and Biological Correlates of Response in Patients with Metastatic Melanoma: Results from a Phase I Trial of Atezolizumab



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Abstract

Purpose: Atezolizumab [anti-programmed death-ligand 1 (PD-L1)] selectively targets PD-L1 to block its interaction with receptors programmed death 1 and B7.1, thereby reinvigorating antitumor T-cell activity. We evaluated the long-term safety and activity of atezolizumab, along with biological correlates of clinical activity endpoints, in a cohort of patients with melanoma in an ongoing phase Ia study (NCT01375842).

Patients and Methods: Patients with unresectable or metastatic melanoma were enrolled to receive atezolizumab 0.1 to 20 mg/kg or ≥ 10 mg/kg every 3 weeks. Primary study objectives were safety and tolerability. Secondary objectives included investigator-assessed efficacy measures; pharmacodynamic and predictive biomarkers of antitumor activity were explored.

Results: Forty-five patients were enrolled and were evaluable for safety. Most treatment-related adverse events (AE)

were grade 1/2 (60%). Fatigue (44%), pruritus (20%), pyrexia (18%), and rash (18%) were the most common treatment-related AEs of any grade. No treatment-related deaths occurred. Overall response rate was 30% among 43 efficacy-evaluable patients, with a median duration of response of 62 months [95% CI, 35–not estimable (NE)]. Clinically meaningful long-term survival was observed, with a median overall survival of 23 months (95% CI, 9–66). Baseline biomarkers of tumor immunity [PD-L1 expression on immune cells, T effector (Teff), and antigen presentation gene signatures) and tumor mutational burden (TMB) were associated with improved response, progression-free survival, and overall survival.

Conclusions: Atezolizumab was well tolerated, with durable responses and survival in patients with melanoma. PD-L1 expression, TMB, and Teff signatures may indicate improved benefit with atezolizumab in these patients.

Introduction

Treatment of metastatic melanoma has advanced significantly in recent years. The introduction of targeted agents has revolutionized treatment and improved survival for patients with advanced disease (1). In addition to targeted therapies, cancer

immunotherapy has provided a substantial clinical benefit in metastatic melanoma. Immune checkpoint inhibitors, such as cytotoxic T lymphocyte antigen 4 (CTLA-4), programmed death 1 (PD-1), and programmed death-ligand 1 (PD-L1) inhibitors, have been shown to improve patient outcomes when used alone or in combination regimens for melanoma treatment (2).

PD-L1 binds to its receptors, PD-1 and B7.1 (CD80), on activated T cells to dampen T-cell immune responses and promote tumor immune escape (3, 4). PD-L1 is expressed on tumor cells (TC) and tumor-infiltrating immune cells (IC) in melanoma and a wide range of other tumor types (5). Combination of PD-L1/PD-1 inhibitors with CTLA-4-targeted agents has yielded higher response rates (6). The PD-1 inhibitors pembrolizumab (as a single agent) and nivolumab (alone or in combination with ipilimumab) have been approved to treat advanced disease.

Tumors with a high mutational burden, such as melanoma, have been shown to respond well to PD-L1/PD-1 inhibition (6–13). Highly mutated tumors display an increased level of tumor-derived neoantigens that render them more susceptible to tumor-specific cytotoxic T-cell [effector T cell (Teff)]-mediated killing (14). T-cell infiltration and the level of PD-L1 expression can make tumors more susceptible to PD-L1/PD-1 inhibitors. Melanoma tumors are some of the most T-cell-infiltrated tumor types, and PD-L1 expression is found on both TC and

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Translational Relevance

Immune checkpoint inhibitors [nivolumab and pembrolizumab (anti-PD-1), ipilimumab (anti-CTLA-4), atezolizumab (anti-PD-L1)], in addition to BRAF- and MEK-targeted therapies (vemurafenib, dabrafenib, trametinib, cobimetinib), have revolutionized the treatment of melanoma. PD-L1/PD-1 inhibitors, in particular, have shown durable responses and potential for long-term survival in patients with melanoma. In this phase I melanoma cohort with long-term follow-up, atezolizumab demonstrated a tolerable safety profile along with durable clinical activity. In addition, PD-L1 expression, tumor mutational burden, and T-effector gene signatures were identified as biomarkers of atezolizumab clinical activity in this patient population. These data may help to identify patients with melanoma and other solid tumors who could benefit from treatment with atezolizumab immunotherapy. Furthermore, biological correlates of long-term clinical activity described here represent a substantial contribution to the understanding of PD-L1/PD-1 inhibition in malignant melanoma.

IC (5, 15–17). These data provide the rationale for therapeutic targeting of PD-L1 in metastatic melanoma.

Atezolizumab is an engineered, humanized anti-PD-L1 mAb that blocks the interaction of PD-L1 with PD-1 and B7.1, thus enhancing tumor-specific T-cell immunity (3, 5). Atezolizumab has demonstrated clinical activity as monotherapy and in combination with other agents in a broad range of tumors, including melanoma, non-small cell lung cancer, and urothelial carcinoma (18–20). Response to atezolizumab was found to be higher in patients with elevated levels of PD-L1 expression on TC or IC (11, 21), although the predictive nature of the PD-L1 biomarker may vary by tumor type or treatment setting, among other considerations (10, 22).

A phase Ia study of atezolizumab was designed to evaluate the safety and activity of atezolizumab in various tumor types and to study the molecular mechanisms associated with clinical benefit via an intensive biopsy-based study protocol. Preliminary results from this study, including the melanoma cohort, were published previously (5). We present the results of long-term follow-up of patients from this cohort and the retrospective evaluation of biomarkers associated with clinical benefit of atezolizumab.

Patients and Methods

Study design and drug treatment

This cohort of patients with metastatic melanoma was one of several enrolled in a larger ongoing phase Ia study (PCD4989g; NCT01375842) evaluating atezolizumab (MPDL3280A) monotherapy in patients with locally advanced or metastatic solid tumors or hematologic malignancies (Supplementary Fig. S1).

Patients were treated with atezolizumab intravenously every 3 weeks. Those with melanoma who were enrolled in the dose-escalation phase of the study received atezolizumab at doses ranging from 0.1 to 20 mg/kg. Additional patients were enrolled and treated in expansion cohorts at doses of ≥ 10 mg/kg. Initially, patients were treated for a maximum of 16 cycles or 1 year,

whichever came first. Patients who experienced a complete or partial response (CR or PR) or had stable disease (SD) were followed every 6 to 12 weeks until loss of clinical benefit, disease progression, or unacceptable toxicity. A later protocol amendment permitted patients to resume treatment if they progressed during follow-up, and a subsequent amendment allowed patients to return to treatment even if they had not progressed, at which point all patients on study were treated until loss of clinical benefit.

The study protocol and amendments were approved by the local institutional review board or ethics committee. The trial was conducted according to the Declaration of Helsinki and International Conference on Harmonisation Guidelines for Good Clinical Practice. All patients provided written informed consent. This study was sponsored by Genentech Inc., a member of the Roche Group, who provided the study drug.

Study assessments

The primary objective of the study was to evaluate the safety and tolerability of atezolizumab. Secondary objectives included assessment of efficacy by the investigator, including overall response rate (ORR), progression-free survival (PFS), overall survival (OS), and duration of response. Selected biomarkers were examined for possible association with response to atezolizumab. The first patient began treatment in August 2011, and last patient began treatment in September 2012. The data cutoff for assessment of safety and efficacy was September 30, 2018; for biomarker analyses, the data cutoff was December 31, 2016, unless otherwise noted.

Patients

Key eligibility criteria for the melanoma cohort included incurable or metastatic uveal, mucosal, or cutaneous melanoma; measurable disease per Response Evaluation Criteria in Solid Tumors (RECIST) v1.1; age ≥ 18 years; Eastern Cooperative Oncology Group performance status of 0 or 1; adequate hematologic and end-organ function; and availability of tumor biopsy specimens at baseline (in dose-expansion cohorts). Individuals were excluded if they had received an approved anticancer therapy, including chemotherapy, hormonal therapy, immunotherapy, or radiotherapy, ≤ 3 weeks before initiation of study treatment; however, hormone replacement therapy or oral contraceptives were allowed. No other concomitant chemotherapy, hormonal therapy, radiotherapy, or investigational agents were permitted. Previous treatment with anti-PD-1 therapy was not permitted. Prior anti-CTLA-4 treatment was initially excluded, but the protocol was later amended to allow it if it had been ≥ 12 weeks from the first dose, > 6 weeks from the last dose, and there was no history of severe immune-mediated related adverse effects. Prior treatment with systemic immunostimulatory agents such as IFN α and IL2 was permitted if completed > 6 weeks or 5 drug half-lives before initiation of the study drug. Patients with primary central nervous system (CNS) malignancy or symptomatic CNS metastases could not participate in the trial, although asymptomatic CNS disease was allowed.

Safety evaluations

Safety data were graded on the basis of the NIH Common Terminology Criteria for Adverse Events, v4.03. All adverse events (AE) were recorded until 90 days after the last administration of

study drug or initiation of another anticancer therapy, whichever came first. Subsequently, only serious AEs, deemed by the investigator to be treatment related, were recorded.

Response assessment

Response assessments were conducted by CT scans using RECIST v1.1, which were performed every 6 weeks for the first 24 weeks and every 12 weeks thereafter. Responses were also assessed by immune-related response criteria (irRC), which is used to account for atypical response patterns, including possible pseudoprogression/influx of immune cells or delayed antitumor activity. Duration of response was analyzed for all responders. PFS and OS were defined as time from date of first dose of atezolizumab to time of disease progression or death, respectively.

Biomarkers

Tumor biomarkers were evaluated in available tissue collected before (archival and/or freshly collected) and after atezolizumab exposure. There was no restriction on the timeframe for collection of archival tissue prior to exposure. The median time from tissue collection to start of atezolizumab was 194 days (range, 0–1,800 days). PD-L1 expression was measured on TC and IC by IHC ($n = 39$), which was performed in a central laboratory on formalin-fixed paraffin-embedded tumor specimens using the Ventana SP142 IHC assay (Ventana Medical Systems). Tumor CD8 IHC (SP16 clone) was quantified on the basis of a continuum ($n = 39$).

Gene expression levels were quantified by TruSeq RNA access RNA-seq (Illumina) in RNA extracted from formalin-fixed ($n = 34$), paraffin-embedded tissues (23). The expression values for each gene were standardized: $z = x - \mu/\sigma$. μ and σ were estimated in the entire input data or the selected subgroups via the subgroup widget; each gene was standardized separately. Following standardization, the values were averaged across genes within each patient. Detection of *BRAF* mutations was performed locally ($n = 32$), whereas other mutations and tumor mutational burden (TMB) were estimated by targeted genomic profiling by Foundation Medicine (ref. 24; $n = 23$). RNA-based gene signatures reflecting the tumor microenvironment (immune, stromal, and cancer related) were used to analyze their involvement in atezolizumab clinical activity (Supplementary Table S1). Gene signatures were validated using the GSDecon package in R based on singular value decomposition to create "eigen-genes," which represent the majority of variance across a gene set (25).

Statistical analysis

Demographics and baseline characteristics were summarized for the overall study population and subgroups. Safety analyses included all patients who received ≥ 1 dose of study treatment, and efficacy analyses included patients who received ≥ 1 mg/kg of study treatment. Two patients received < 1 mg/kg of atezolizumab. The estimated ORR for efficacy-evaluable patients and 95% CIs were calculated using the Clopper–Pearson method. Comparisons of ORR between two subgroups were performed using the Fisher exact test. The Kaplan–Meier method was used to estimate PFS and OS; the log-rank test was performed for subgroup comparisons, with HRs estimated from the Cox model. Differential expression analysis of gene signatures between the clinical response groups was performed using Wilcoxon rank-sum tests.

Because of the exploratory nature of the analysis, only nominal *P* values were reported.

Data availability

Qualified researchers may request access to individual patient-level data through the clinical study data request platform (www.clinicalstudydatarequest.com). Further details on Roche's criteria for eligible studies are available here (<https://clinicalstudydatarequest.com/Study-Sponsors/Study-Sponsors-Roche.aspx>). For further details on Roche's Global Policy on the Sharing of Clinical Information and how to request access to related clinical study documents, see here (https://www.roche.com/research_and_development/who_we_are_how_we_work/clinical_trials/our_commitment_to_data_sharing.htm).

Results

Patient demographics and baseline characteristics

As of the data cutoff of September 30, 2018, 45 patients with melanoma were enrolled in the study; all 45 patients were evaluable for safety. Two patients were not evaluable for efficacy. The median follow-up was 75 months (range, 1–79 months). Patient demographics and baseline characteristics for the safety-evaluable population are shown in Table 1. Eighty percent of patients had cutaneous, 9% had uveal, and 11% had mucosal melanoma.

Safety

The median duration of atezolizumab therapy was 5 months (range, 0–69 months). Treatment duration ranged from 0 to < 3 months in 40% of patients, ≥ 3 to < 6 months in 18%, ≥ 6 to < 12 months in 27%, and ≥ 12 months in 16%. The median number of doses received was 7 (range, 1–96 doses). AEs leading to dose modification or interruption occurred in 8 patients (18%).

In general, the observed AE profile was consistent with that seen in other studies of single-agent atezolizumab and in other cohorts in this study. All-cause and treatment-related AE (TRAE) data are summarized in Supplementary Table S2. Most TRAEs were grade 1/2 in severity and were considered manageable and reversible. Grade 3/4 TRAEs were increased alanine aminotransferase (ALT; 4%) levels, and autoimmune hepatitis, hyperbilirubinemia, increased γ -glutamyl transferase levels, increased lipase levels, increased aspartate aminotransferase (AST) levels, decreased lymphocyte count, hypoxia, fatigue, and generalized erythema (each 2%). A total of 4 treatment-related serious AEs occurred in 3 patients (7%), including 3 grade 3/4 events (grade 3 hypoxia, grade 4 increased ALT levels, and grade 4 increased AST levels). TRAEs leading to treatment modification or interruption occurred in 4 patients (9%).

Four patients (9%) had AEs that led to treatment withdrawal, including 3 grade 3 AEs (increased lipase levels, autoimmune hepatitis, and hypoxia) and 2 grade 4 AEs (increased ALT and AST levels). AEs of special interest occurring in $> 5\%$ (all grades) of patients were rash (20%), maculopapular rash (13%), vitiligo, increased ALT levels and increased AST levels (9% each), and hypothyroidism (7%). No dose-limiting toxicities were noted, and no treatment-related deaths occurred.

Any-grade TRAEs reported over time with atezolizumab therapy are shown in Supplementary Table S3 (≤ 1 year of exposure, 78%; 1–2 years, 43%; 2–3 years, 50%; ≥ 3 years,

Table 1. Patient demographics and baseline characteristics

Demographic or disease characteristics	All doses (N = 45) ^a
Median age (range), y	63.0 (21-83)
Male, n (%)	30 (66.7)
ECOG performance status 0/1/2, %	77.8/20.0/2.2
Histology type, n (%) ^b	
Cutaneous	35 (79.5)
Mucosal	5 (11.4)
Uveal	4 (9.1)
Stage at initial diagnosis, n (%) ^c	
IA	2 (4.4)
IIA	4 (8.9)
IIB	4 (8.9)
IIC	4 (8.9)
IIIA	6 (13.3)
IIIB	3 (6.7)
IIIC	3 (6.7)
IV	14 (31.1)
Stage at screening, n (%) of patients with cutaneous histology	
M1a	5 (14)
M1b	5 (14)
M1c	25 (71)
LDH elevated ($\geq 1.5 \times$ ULN), n (%)	7 (15.6)
BRAF status, n (%)	
Mutation detected	11 (24.4)
No mutation detected	21 (46.7)
Unknown	13 (28.9)
Prior systemic regimens for metastatic disease, n (%), median	
0	18 (40.0)
1	9 (20.0)
2	11 (24.4)
3	3 (6.7)
≥ 4	4 (8.9)
Most common prior systemic therapies, n (%) ^d	
Immunotherapy	14 (31.1)
Chemotherapy	12 (26.7)
IL2	10 (22.2)
Cytokine	9 (20.0)
BRAF inhibitor ^e	6 (13.3)
MEK inhibitor	5 (11.1)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; IL2, interleukin 2; LDH, lactate dehydrogenase; ULN, upper limit of normal.

^aSafety-evaluable patients.

^bAmong 44 patients with known histology type.

^cAmong 40 patients with known stage at diagnosis.

^dFor metastatic disease.

^eFour patients treated with BRAF inhibitor therapy were also treated with MEK inhibitor therapy.

40%). The most common any-grade TRAEs in patients treated with atezolizumab for ≤ 1 year ($n = 45$) were fatigue (40%, $n = 18$) and pruritus and pyrexia (18%, $n = 8$ each). In patients treated for ≥ 3 years ($n = 5$), pruritus, diarrhea, arthritis, lymphadenopathy, perivascular dermatitis, and pruritic rash (20%, $n = 1$ for each) were the most common TRAEs of any grade. Eight patients (18%) reported grade 3/4 events with ≤ 1 year of atezolizumab exposure; no grade 3/4 TRAEs were reported beyond the 1-year time point.

Efficacy

Of the 43 patients evaluable for efficacy, there were 13 investigator-assessed confirmed responses per RECIST v1.1, for an ORR of 30.2% (Table 2). Four patients (9%) had SD (≥ 24 weeks) as best response, resulting in a disease control rate (CR + PR + SD ≥ 24 weeks) of 40% (95% CI, 25%-56%). Change in tumor burden over time is depicted in Fig. 1A. Duration of response ranged from

Table 2. ORR in ITT and histologic subgroups

	Patients (N = 43) ^a			
	ITT (N = 43)	Cutaneous (n = 33)	Mucosal (n = 5)	Uveal (n = 4)
ORR, n (%) ^b	13 (30.2)	12 (36.4)	1 (20.0)	0
95% CI	17.18-46.13	20.40-54.88	0.51-71.64	0.00-60.24
CR, n (%)	4 (9.3)	4 (12.1)	0	0
PR, n (%)	9 (20.9)	8 (24.2)	1 (20.0)	0
SD, n (%)	11 (25.6)	9 (27.3)	0	1 (25.0)
PD, n (%)	18 (41.9)	11 (33.3)	4 (80.0)	3 (75.0)
Missing or unevaluable	1 (2.3)	1 (3.0)	0	0

Abbreviations: ITT, intent to treat; PD, progressive disease.

^aEfficacy-evaluable patients with measurable disease at baseline.

^bInvestigator-assessed best confirmed ORR by RECIST v1.1.

2.8 to 72.7+ months, with a median duration of response of 62 months [95% CI, 35-not estimable (NE)]. Response rates by RECIST v1.1 and irRC were identical; however, an additional 4 patients achieved SD according to irRC (35% by irRC vs. 26% by RECIST v1.1). As of the last tumor data cutoff, 6 of 13 responses were still ongoing (Fig. 1B). Some of these responses were sustained for > 3 years ($n = 4$) following last treatment.

Responses were noted in patients with cutaneous (36%; $n = 12$) and mucosal melanoma (20%; $n = 1$). No responses were observed in patients with uveal melanoma ($n = 4$); 1 patient (25%) had SD. The duration of SD in this patient was 4.9 months, and the best change in the sum of longest diameters was 9%. Patients who had received prior IFN α or IL2 immunotherapy had a numerically higher response rate and a nonsignificant trend toward longer PFS and OS than those without prior immunotherapy [ORR, 41% ($n = 7/17$) vs. 23% ($n = 6/26$); PFS HR, 0.64 (95% CI, 0.33-1.17); OS HR, 0.59 (95% CI, 0.29-1.27)]. No patients had received prior anti-CTLA-4 therapy.

Median PFS (mPFS) was 4 months (range, 1-75+ months) among all efficacy-evaluable patients and 6 months (range, 1-75+ months) for those with cutaneous melanoma. Landmark analyses indicated 6-month and 1-year PFS rates of 37% (95% CI, 22-52) and 33% (95% CI, 19-47), respectively (Fig. 1C).

Median follow-up for OS was 75 months (range, 1-79 months). For all efficacy-evaluable patients, median OS (mOS) was 23 months (95% CI, 9-66). The Kaplan-Meier-estimated 1-year survival rate for all patients was 58% (95% CI, 43%-74%; Fig. 1D). At 2, 3, and 4 years, OS rates for all patients were 48% (95% CI, 33%-64%), 40% (95% CI, 25%-56%), and 38% (95% CI, 22%-53%), respectively. Seventeen patients (40%) were still alive at data cutoff.

Baseline biomarkers associated with clinical activity

PD-L1 and tumor immune infiltration. PD-L1 expression on IC and TC has been associated with atezolizumab clinical activity in solid tumors (5, 26). Thirty-nine of 43 patients were evaluated for PD-L1 IHC status using the Ventana SP142 assay. Five of the 39 patients (12%) expressed PD-L1 on $\geq 1\%$ of TC, and 22 of the 39 (56%) expressed PD-L1 on $\geq 1\%$ of IC (Supplementary Table S4). One of 17 patients with PD-L1 expression on $< 1\%$ of IC received 0.1 mg/kg atezolizumab and was excluded from the efficacy-evaluable population ($n = 38$). Expression of PD-L1 on TC was not associated with atezolizumab clinical outcomes. ORR was

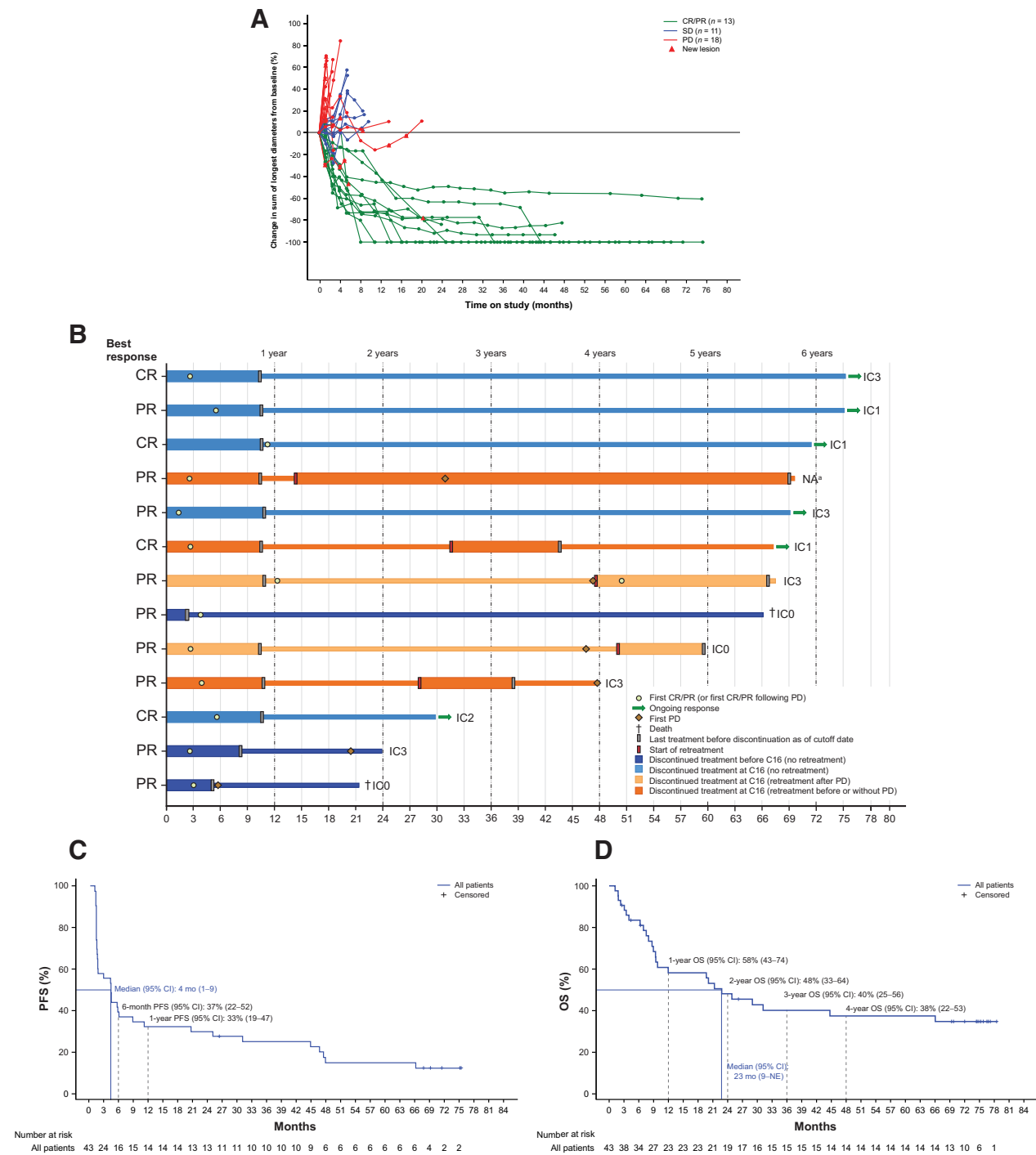


Figure 1. Clinical activity of atezolizumab in patients with melanoma. **A**, Change in tumor burden over time. Patients without postbaseline tumor assessments were excluded. **B**, Duration of response in patients with objective responses. **C**, PFS with 6- and 12-month landmarks. **D**, OS with 1- to 4-year landmarks. Investigator-assessed confirmed responses per RECIST v1.1 are shown. Data cutoff, September 30, 2018. Patients were first treated with atezolizumab 1–20 mg/kg by July 1, 2013. NA, not applicable; SLD, sum of the longest diameters. ^aPD-L1 IC status unknown.

20% ($n = 2/5$) and 26% ($n = 9/34$) in patients with PD-L1 expression on $\geq 1\%$ and $< 1\%$ of TC, respectively, while the HRs for PFS and OS in patients with $\geq 1\%$ PD-L1 TC expression were 0.94 (95% CI, 0.36–2.48) and 0.51 (95% CI, 0.19–1.80), respec-

tively. Of note, all responding patients with PD-L1 expression on $\geq 1\%$ of TC also had PD-L1 expression on $\geq 1\%$ of IC. An association between PD-L1 expression on IC and response to atezolizumab was seen. As of September 30, 2018, 3 of 16 patients

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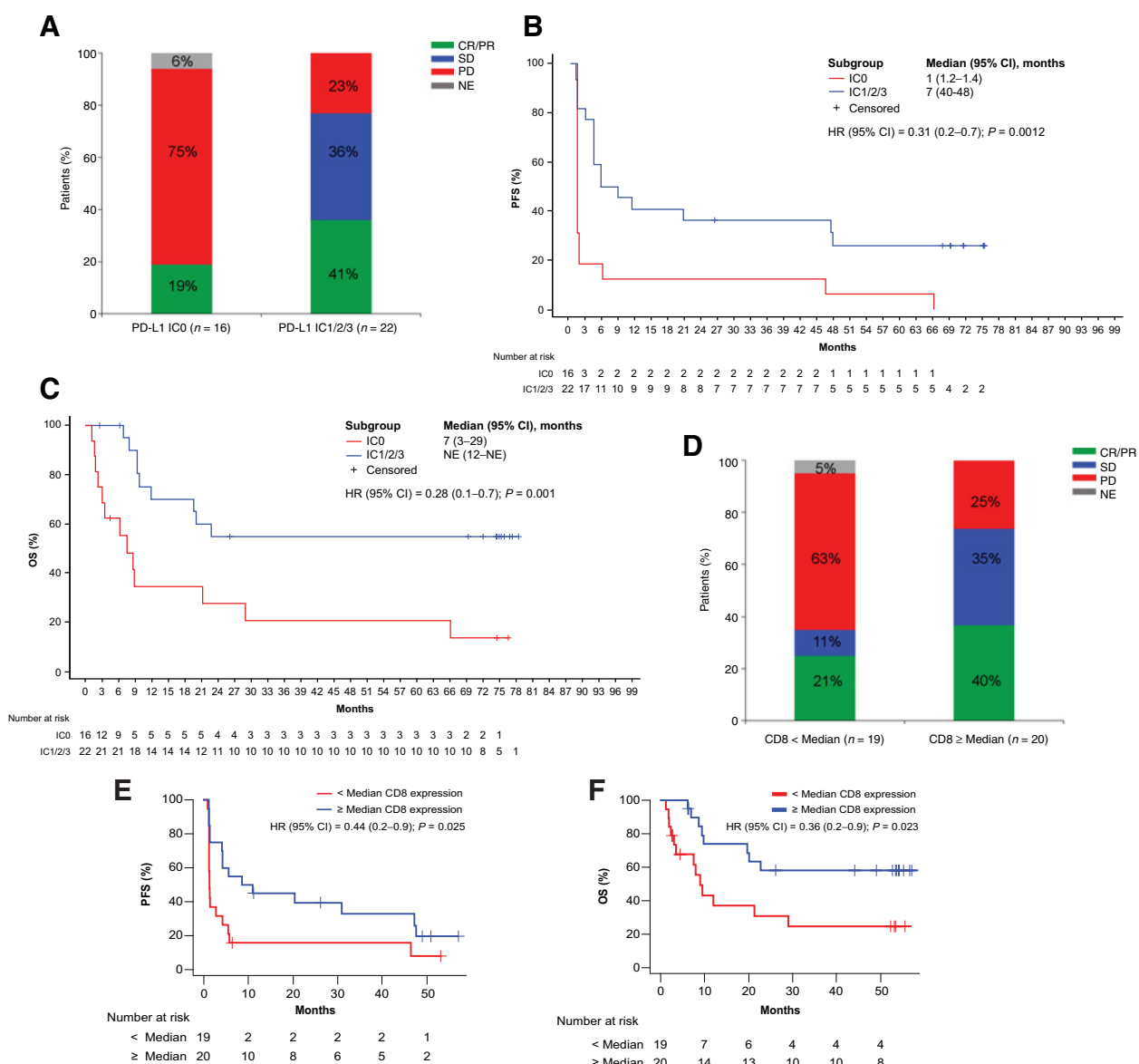


Figure 2. Atezolizumab clinical outcome by PD-L1 IC and tumor-infiltrating CD8⁺ T-cell IHC. **A**, ORR. **B**, PFS. **C**, OS by PD-L1 status (PD-L1 IC0 vs. IC1/2/3). One of the 17 patients with IC0 PD-L1 IHC status received < 1 mg/kg atezolizumab and was excluded from the efficacy-evaluable population. **D**, ORR. **E**, PFS. **F**, OS by median CD8 IHC expression (median = 2.07% of CD8 staining as percentage of tumor area).

(19%) with PD-L1 IC0 tumors achieved PR, and 9 of 22 patients (41%) with PD-L1 IC1/2/3 tumors achieved CR ($n = 4$) or PR ($n = 5$). No patient with PD-L1 IC0 tumors versus 8 of 22 patients (36%) with PD-L1 IC1/2/3 tumors had SD (Fig. 2A). PFS and OS were also increased in patients with PD-L1 expression on IC. mPFS was longer in patients with PD-L1 IC1/2/3 tumors [7 months (95% CI, 4–48 months)] compared with those with PD-L1 IC0 tumors [1 month (95% CI, 1.2–1.4 months)], with an HR of 0.31 (95% CI, 0.2–0.7; $P = 0.0012$; Fig. 2B), and mOS was longer in patients with PD-L1 IC1/2/3 tumors [NE (95% CI, 12 months–NE)] compared with those with PD-L1 IC0 tumors [7 months (95% CI, 3–29 months)] with an HR of 0.28 (95% CI, 0.1–0.7; $P = 0.002$; Fig. 2C). These data indicate that patients

harboring PD-L1-expressing IC are linked to atezolizumab clinical outcomes.

The presence of CD8⁺ cytotoxic T cells has been linked to improved activity of checkpoint inhibitors in melanoma (16). CD8 immunostaining was performed in 39 patients, with a median expression of 2.22% as the percentage of tumor center area. Patients with more than the median level of CD8 expression had improved clinical response [ORR, 40% (95% CI, 6%–46%) vs. 21% (95% CI, 19%–64%)], mPFS [9 months (95% CI, 1–48 months) vs. 1 month (95% CI, 1–4 months); $P = 0.025$], and mOS [not reached (NR; 95% CI, 10 months–NR) vs. 9 months (95% CI, 3–29 months); $P = 0.023$] compared with those with CD8 levels below the median (Fig. 2D–F).

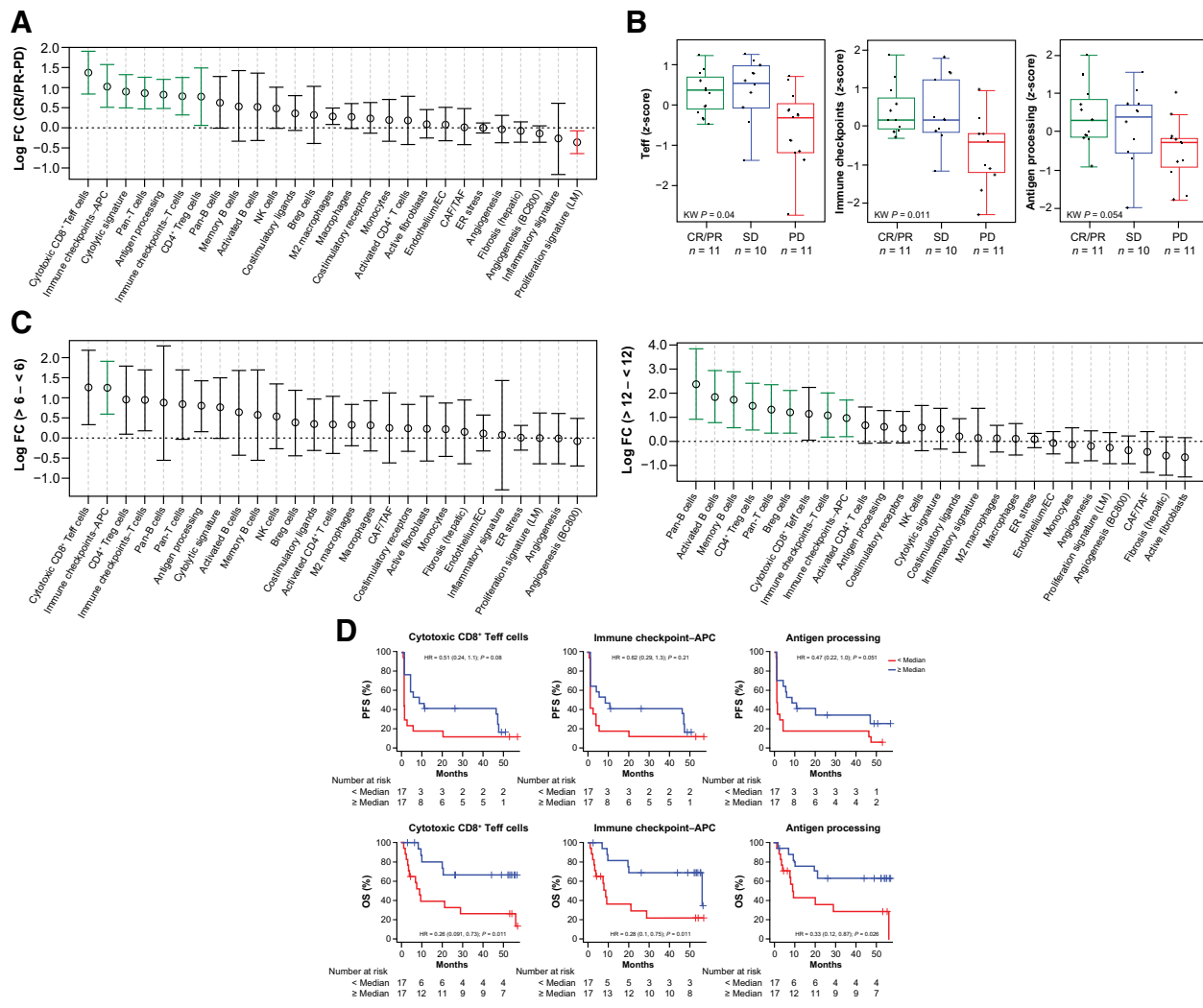


Figure 3.

Association of immune, tumor, and stromal gene signature biomarkers and outcomes. **A**, Summary of gene set activity in CR/PR versus PD patients for the immune signature collection. For each pathway, the average fold change and 95% CIs are plotted. Colors correspond to significance according to raw *P* values when fold change is compared with zero (green = significantly higher in CR/PR; red = significantly lower in CR/PR). **B**, Expression of gene signatures for cytotoxic CD8⁺ T cells, immune checkpoint APC, and antigen processing by response. Boxes represent the median and interquartile range (lower quartile, 25%; upper quartile, 75%), and the whiskers show the range of gene expression. KW *P* values: Kruskal-Wallis one-way analysis. **C**, Differential expression of RNA-based gene signatures between patients with PFS events before or after 6 months (top graph) and with death events before or after 12 months (bottom graph) of atezolizumab exposure. **D**, PFS and OS by cytotoxic CD8⁺ T cells, immune checkpoint APC, and antigen-processing gene signatures. APC, antigen-presenting cells; FC, fold change difference; PD, progressive disease.

Different aspects of tumor biology, such as immune infiltration, stroma, and cancer-associated pathways, have been linked to atezolizumab monotherapy clinical outcomes. To address the role of these biologies in our melanoma cohort ($n = 34$), we evaluated different biological pathways using RNA-based gene signatures (Supplementary Table S1; ref. 23). These gene signatures were used to compare patients who responded to atezolizumab (CR/PR, $n = 11$) with those who experienced progression ($n = 11$). Consistent with the immunostaining results, the 6 signatures associated with response were related to T-cell biology and checkpoint inhibitors (cytotoxic CD8⁺ T cells, immune checkpoint antigen-presenting cells, cytolytic signature, pan-T cells, antigen processing, immune checkpoint T cells); only 1 signature was associated with disease progression and reflected

tumor cell-intrinsic biology (cell-cycle proliferation; Fig. 3A). Ten patients who had a best response of SD were also included in the analysis. Interestingly, patients with SD had similar expression patterns as responders (Fig. 3B), suggesting that the gene signatures are associated with clinical benefit (CR/PR/SD).

To address the impact of these different biologies on long-term clinical outcome, we determined the association of the signatures with 6-month PFS and 12-month OS rates. Consistent with the above findings, gene signatures associated with better PFS were characteristic of T-cell biology and immune checkpoint inhibitors, particularly cytotoxic CD8⁺ T cells and immune checkpoints/antigen-presenting cells (Fig. 3C). These same gene signatures, as well as those associated with B-cell biology, were associated with longer OS. Using a median expression cutoff,

these gene signatures were found to be significantly associated with PFS and OS (Fig. 3D).

Genomic alterations and atezolizumab clinical outcome. To evaluate the association between atezolizumab activity and relevant melanoma markers, we examined response to atezolizumab by *BRAF* mutation status. Thirty-two of 43 biomarker-evaluable patients had *BRAF* V600 mutation status reported: 11 (34%) had *BRAF* mutations (V600E, $n = 10$; V600K, $n = 1$) and 21 (66%) had wild-type *BRAF*. No significant differences in ORR, PFS, or OS were observed with respect to *BRAF* mutation status. ORR was 30% ($n = 3/10$) and 29% ($n = 6/21$) in *BRAF* mutation-positive and wild-type patients, respectively, while the HRs for PFS and OS in *BRAF* mutation-positive patients were 1.29 (95% CI, 0.57–3.09) and 1.34 (95% CI, 0.49–3.88), respectively (data cutoff: September 30, 2018). In this limited dataset, no responses were observed in the 6 patients with *BRAF*-mutated melanoma who were previously treated with a *BRAF* inhibitor.

In addition to tumor PD-L1 associated with clinical benefit from checkpoint inhibitors, TMB and tumors harboring DNA damage repair (DDR) have been associated with enrichment for clinical benefit from checkpoint inhibitors (8, 12, 23, 27–30). The TMB cutoff of 16 mutations/Mb, as assessed by the FoundationOne assay, was associated with increased benefit from atezolizumab in patients with non-small cell lung cancer and metastatic urothelial carcinoma (8, 12, 23, 29, 31). Of the 23 patients assessed for TMB in our study, 11 (47%) had < 16 mutations/Mb (TMB low) and 12 (53%) had \geq 16 mutations/Mb (TMB high). Clinical activity was higher in TMB-high patients than in TMB-low patients: ORR was 50% ($n = 6/12$) versus 0% ($n = 0$ of 11; Fig. 4A), mPFS was 20 months vs 1 month [HR, 0.13 (95% CI, 0.03–0.47)], and mOS was NR versus 7 months [HR, 0.07 (95% CI, 0.01–0.32)], respectively (Fig. 4B and C). TMB was also associated with biomarkers of immune infiltration, such as PD-L1 on both IC and TC (IC, $r = 0.71$; TC, $r = 0.40$) and tumor-infiltrating CD8⁺ T cells ($r = 0.51$; Fig. 4D).

Nine patients harboring mutations in genes involved in the DDR pathway had increased TMB versus 13 patients with non-DDR-mutated tumors (mean TMB, 49 ± 25 mutations/Mb vs 17 ± 18 mutations/Mb; Supplementary Fig. S2A and S2B). Patients with DDR-mutated tumors had increased ORR and longer PFS and OS compared with patients with DDR-proficient tumors (Supplementary Fig. S2C–S2E).

Genomic alterations of *JAK1* and *PTEN/PIK3CA* pathways. Loss of function in the IFN γ signaling pathway, particularly *JAK1/JAK3/STAT1*, or activating alterations in the *PTEN/PIK3CA* pathway have been associated with mechanisms of resistance to checkpoint inhibitors (32, 33). In this study, 1 patient had a tumor with a missense mutation in *JAK1* (R659H, unknown biological significance), 2 patients had tumors with genomic loss of *PTEN*, and 1 patient had a tumor with an activating mutation in *PIK3CA* (Q546K). The patients carrying *JAK1* mutations and *PTEN* genomic loss experienced disease progression, whereas the patient with a *PIK3CA*-activating mutation had SD. Neither *PTEN* loss nor *PIK3CA* activation were linked to reduced OS because the patients with these alterations were alive at the time of analysis (52.2+ and 52.7+ months). The data for the patients with these genomic

alterations are consistent with reports associating them with lack of response.

Pseudoprogression in a TMB-high/immune infiltration–low patient

A 59-year-old male patient with *BRAF*-mutated (V600K) cutaneous melanoma experienced signs of pseudoprogression upon atezolizumab exposure (Supplementary Fig. S3). A scan on day 35 showed enlargement of a cutaneous lesion, resulting in a sum of the longest diameter change of +31%; the lesion looked necrotic and a biopsy was collected. On day 77, the lesion decreased to +6% and continued to shrink. On day 101, the lesion was fully resected for diagnostic purposes, and since then, the patient has had no evidence of disease. Other functional mutations detected in this patient were single-nucleotide variations in *IDH1* (R132C) and genomic loss of *PTEN* and *CDKN2B*, and *CDKN2A*. Consistent with *PTEN* loss in a prior report (33), the patient's pretreatment sample had minimal signs of immune cell infiltration (PD-L1 IC, 0%; PD-L1 TC, 0%; CD8, 0.12%); however, robust infiltration was detected at the time of pseudoprogression (PD-L1 IC, 15%; PD-L1 TC, 25%; CD8, 3%) and at resection of the tumor lesion (PD-L1 IC, 15%; PD-L1 TC, 2%; CD8, 5%; Supplementary Fig. S3A). TMB in the pretreatment and posttreatment tissue was elevated (22 mutations/Mb and 19 mutations/Mb, respectively; Supplementary Fig. S3B). This patient demonstrated that despite lack of preexisting immune infiltration, a highly mutated tumor is still able to trigger an effective antitumor immune response upon atezolizumab exposure.

Characterization of long-term responders

At the time of biomarker data cutoff (December 31, 2016), 6 of 12 responders had an ongoing response [median duration of response, 38+ months (range, 3+–52+ months)], whereas the remaining 6 responders had progressed [median duration of response, 32 months (range, 3–44 months)]. Three of the ongoing responders had CR, whereas all the patients who stopped responding had PR. Of > 300 molecular and demographic parameters evaluated, body mass index (BMI) was the only parameter associated with ongoing response. The median BMI for ongoing responders was 33 kg/m² (range, 26–33 kg/m²) versus 23 kg/m² (range, 20–32 kg/m²) for nonongoing responders (Supplementary Fig. S4). Of note, a baseline BMI indicative of overweight/obesity was not significantly associated with increased ORR, PFS, or OS in this cohort [ORR, 36% (BMI > 30, $n = 5/14$) vs. 25% (BMI < 30, $n = 7/28$); PFS HR, 0.52 (95% CI, 0.27–1.01); OS HR, 0.72 (95% CI, 0.33–1.61); data cutoff: September 30, 2018].

Discussion

This phase Ia study found that atezolizumab was well tolerated and provided clinical benefit in patients with metastatic melanoma. The observed AE profile was consistent with that seen in other single-agent atezolizumab studies and in the other cohorts from this phase Ia study (5, 13, 21, 34). In addition, the observed toxicity profile was comparable to that seen in the preliminary analysis, suggesting that there is not a significant rate of new AEs arising over time (5). Indeed, the incidence of TRAEs decreased after the first year of atezolizumab exposure. Fatigue, diarrhea, and pruritis were among the AEs reported with \geq 2 years of exposure.

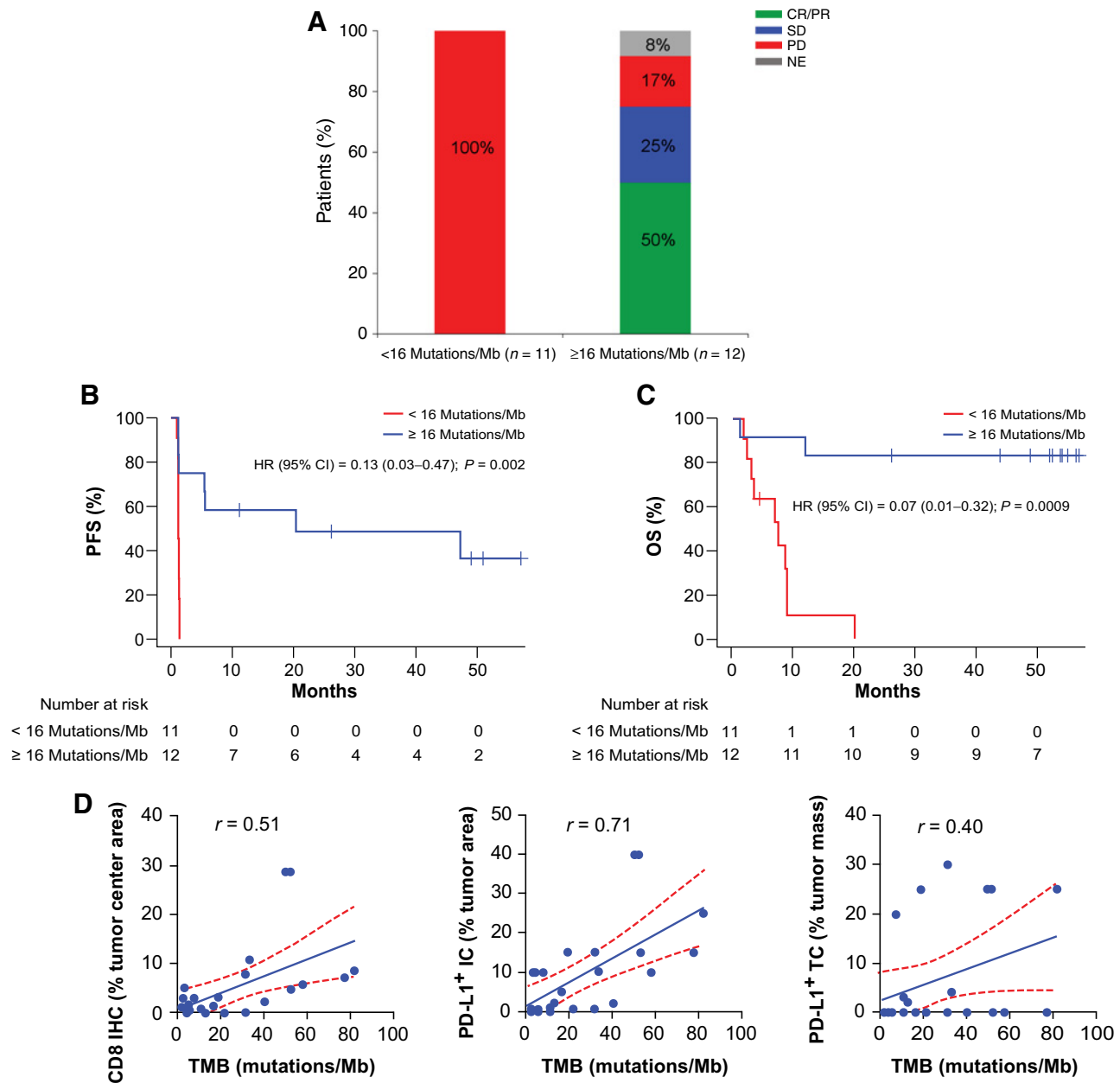


Figure 4. Atezolizumab clinical outcome by TMB. **A**, Response by TMB at < or ≥ 16 mutations/Mb. **B**, PFS. **C**, OS by TMB at < or ≥ 16 mutations/Mb. **D**, Correlation between TMB and CD8 or PD-L1 IC and TC.

Efficacy was observed in patients with or without prior systemic immunotherapy. Moreover, responses were observed in both *BRAF*-mutant and wild-type patients and in cutaneous and mucosal histologies, similar to responses seen with PD-1 inhibitors (35, 36). Responses to atezolizumab were durable, with some patients continuing treatment > 4 years after treatment initiation. The median duration of response was 62 months, and 6 of 13 responses are still ongoing. Notably, these 6 patients have sustained responses despite no longer receiving treatment. In addition, of the 16 patients who lived ≥ 3 years, 3 had progressive disease as best response, suggesting a potential prolonged clinical

benefit of atezolizumab in these patients despite a lack of radiographic response, although information on subsequent therapies was not collected.

Clinically meaningful long-term survival was seen with atezolizumab, with a median follow-up of > 6 years (75 months). The landmark OS rates are similar to those reported with other checkpoint inhibitors. Long-term follow-up of a phase I trial in previously treated patients with advanced melanoma who received nivolumab for ≤ 2 years reported an mOS of 17 months (95% CI, 13–38 months), with 1-year, 3-year, and 5-year survival rates of 63%, 42%, and 34%, respectively (37). Similar results

were also seen in a phase I study of pembrolizumab, which showed 4- and 5-year OS rates of 38% and 34%, respectively (38). Long-term follow-up in this study approaches that of the others and suggests that atezolizumab also can provide long-term control of metastatic melanoma. However, the number of patients with elevated LDH in this study was low (~16%), thus limiting potential cross-trial survival comparisons with other PD-1–targeting agents that may have enrolled higher or lower risk patients. Furthermore, information on subsequent immunotherapy or other anticancer therapy was not collected for patients who experienced progressive disease while receiving atezolizumab. Thus, no conclusion can be made as to the effect of these treatments, including any immunotherapies, on the OS of this cohort. Treatment with immunotherapy before atezolizumab exposure was associated with a numerically higher ORR and a trend for longer survival. Immune biomarkers analyzed in the tumors of these patients were similar to those in patients who did not receive prior immunotherapies, partially explaining the lack of statistical significance of this condition and survival (PFS, OS). However, it should be noted that these were not preplanned subset analyses; thus, any statistical comparisons are for descriptive purposes only.

The presence of immune checkpoints and CD8⁺ T cells, detected either by RNA expression or immunostaining, was associated with improved clinical outcomes in this study. The increased response rate and survival in patients with higher PD-L1 IC levels suggest that PD-L1 expression on IC might serve as a predictive biomarker of response to atezolizumab in melanoma, as observed in some other tumor types and treatment settings (8, 10–12, 21, 22). An analysis of phase I to III trials of PD-L1 and PD-1 inhibitors in melanoma found a 23% absolute increase in ORR for PD-L1–positive versus PD-L1–negative tumors (49% vs. 26%; $P < 0.0001$; ref. 39). In addition to PD-L1, other baseline clinical parameters, such as relative eosinophil and lymphocyte counts, lactate dehydrogenase levels, and absence of metastasis other than soft-tissue or lung, are being evaluated as possible predictors of OS in patients with melanoma treated with immune checkpoint inhibitors (40). Similar analyses are ongoing within this trial.

Analysis of TMB showed that patients with tumors that had a TMB of ≥ 16 mutations/Mb derived higher clinical benefit from atezolizumab than patients with low TMB. There was a significant association between elevated TMB and defects in genes belonging to the DDR machinery, pointing to this as a mechanism for elevated mutation rate. Increased mutational frequency correlated with biomarkers of immune infiltration to different degrees. In the case of the patient experiencing pseudoprogression, the pretreatment tumor sample had no immune infiltration but did have high TMB. CD8 and PD-L1 expression increased upon atezolizumab exposure, suggesting that TMB may be linked to increased neoantigen levels, and this could be sufficient to allow for the reactivation of tumor-reactive cells by atezolizumab, triggering an effective antitumor immune response.

Among several biologies representing immune cells, stroma, or the tumor, those that correlated best with response, PFS, and OS were the presence of CD8⁺ T cells and PD-L1. B cells have been linked to better prognosis in highly immunogenic tumors (41, 42). Because B-cell–associated signatures were linked only to survival and not to response or PFS, we hypothesize that the presence of B cells in this cohort might be prognostic (43, 44). Innate anti–PD-1 resistance gene signatures, which are generally

linked to stromal biology, were not associated with disease progression on atezolizumab, although the disparity with published results could be due to the small number in this study (45).

Activation of the PTEN/PIK3CA pathway and loss of IFN γ /JAK1/JAK3/STAT1 signaling and antigen presentation have been reported as mechanisms of resistance to checkpoint inhibitors in melanoma (32, 33, 46). Although patient numbers were limited in this study, none of the 3 patients carrying activation of the PTEN/PIK3CA pathway responded to atezolizumab. However, these patients were alive at the time of analysis, suggesting that neither *PTEN* loss nor *PIK3CA* activation were linked to reduced OS. Thus, it is possible that these patients might still derive benefits from immunotherapy. Our RNA-based data for antigen-processing signatures are consistent with the findings that antigen processing and presentation is linked to increased activity of checkpoint inhibitors. Because of the unknown biological significance of the *JAK1* mutation, it remains to be addressed whether this alteration is a potential mechanism of resistance to atezolizumab.

Obese male patients (BMI > 30) with melanoma treated with targeted and immune therapies have shown longer PFS and OS than their lean counterparts (47). Although there was only a mild trend in our small cohort, it was surprising that our results suggested that responding overweight/obese patients were more likely to have ongoing responses. Relatedly, pre-clinical models of diet-induced obesity showed increases in the severity of T-cell–mediated autoimmunity and allograft rejection, possibly due to the metabolic reprogramming of T cells (48, 49).

Limitations of this study include its single-arm design and relatively small patient population. Furthermore, the biomarker analyses, although interesting, were exploratory in nature. Despite these limitations, the reported data provide valuable insights into the long-term (median follow-up, 75 months) safety and activity of atezolizumab in patients with metastatic melanoma and contribute to understanding of PD-L1/PD-1 inhibition in this patient population.

In conclusion, atezolizumab appears to be a tolerable and effective treatment option for patients with advanced melanoma, with no grade 3/4 AEs after 1 year and long-term durable responses. The longer follow-up in this trial further supports the previously reported survival benefit seen in this study. Combining immune checkpoint inhibitors with agents that target other steps of the cancer-immunity cycle or small-molecule inhibitors directed against driver oncogenic mutations might enhance antitumor efficacy versus monotherapy. Thus, atezolizumab is a rational partner for combinations with other immunotherapeutics or targeted agents for metastatic melanoma. Studies are continuing to evaluate the efficacy and safety of atezolizumab in combination regimens and to determine potential biomarkers of response.

Disclosure of Potential Conflicts of Interest

O. Hamid reports receiving speakers bureau honoraria from Amgen, Array, Bristol-Myers Squibb, Novartis and Sanofi Regeneron, and is a consultant/advisory board member for Amgen, Bristol-Myers Squibb, Merck, Novartis, and Roche. L. Molinero has ownership interests (including patents) in F. Hoffman-La Roche Ltd. C.R. Bolen has ownership interests (including patents) in Roche. J.A. Sosman is a consultant/advisory board member for Bristol-Myers Squibb and Genentech, reports receiving commercial research grants from Bristol-Myers Squibb and reports receiving commercial research support from Merck. E. Muñoz-Couselo reports receiving speakers bureau honoraria from and is a

consultant/advisory board member for Bristol-Myers Squibb, PIERRA-FABRE, Novartis, MSD, and Roche. H.M. Kluger is a consultant/advisory board member for Alexion, Corvus, Nektar, Bodesix, Genentech, Pfizer, Iovance, Immunocore, Celld, Array Biopharma and Prometheus, and reports receiving commercial research grants from Merck, Bristol-Myers Squibb and Apexigen. D.F. McDermott is a consultant/advisory board member for Merck and Bristol-Myers Squibb. J.D. Powderly is an employee of BioCytics Inc., has ownership interests (including patents) in Biocytics, Iovance, Juno Therapeutics, BlueBird, and Ziopharm, reports receiving speakers bureau honoraria from Bristol-Myers Squibb, Merck and Genentech, is a consultant/advisory board member for Bristol-Myers Squibb, Genentech, Merck, AstraZeneca and Curis, and reports receiving commercial research grants from Genentech, Bristol-Myers Squibb, EMD Serono, AstraZeneca, MacroGenics, InCyte, Arcus, FLX Biosciences, Top Alliance, Alkermes, Tempes, Curis, Corvus, and Abbvie. M. Ballinger and C. O'Hear have ownership interests in Roche. P.S. Hegde reports receiving commercial research support from Genentech. F.S. Hodi has ownership interests (including patents) at Apricity and MICA (to institution), is a consultant/advisory board member for Merck, EMD Serono, Sanofi, Novartis, Takeda, Compass, Bayer, Aduro, Partners, Pfizer, Verastem, and Rheos, and reports receiving commercial research grants from Bristol-Myers Squibb (to institution) and Novartis (to institution). No potential conflicts of interest were disclosed by the other authors.

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References

- Abdul-Karim RM, Cowey CL. Challenging the standard of care in advanced melanoma: focus on pembrolizumab. *Cancer Manag Res* 2017;9:433–42.
- Hepner A, Salgues A, Anjos CAD, Sahade M, Camargo VP, Garicochea B, et al. Treatment of advanced melanoma—a changing landscape. *Rev Assoc Med Bras* 2017;63:814–23.
- Chen DS, Irving BA, Hodi FS. Molecular pathways: next-generation immunotherapy—*inhibiting programmed death-ligand 1 and programmed death-1*. *Clin Cancer Res* 2012;18:6580–7.
- Chen DS, Mellman I. Oncology meets immunology: The cancer-immunity cycle. *Immunity* 2013;39:1–10.
- Herbst RS, Soria JC, Kowanetz M, Fine GD, Hamid O, Gordon MS, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature* 2014;515:563–7.
- Wolchok JD, Chiarion-Sileni V, Gonzalez R, Rutkowski P, Grob JJ, Cowey CL, et al. Overall survival with combined nivolumab and ipilimumab in advanced melanoma. *N Engl J Med* 2017;377:1345–56.
- Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. *N Engl J Med* 2015;372:2018–28.
- Kowanetz M, Zou W, Shames DS, Cummings C, Rizvi N, Spira AI, et al. Tumor mutation load assessed by FoundationOne (FM1) is associated with improved efficacy of atezolizumab (atezo) in patients with advanced NSCLC. *Ann Oncol* 2016;27(suppl 6): 77P.
- Yarchoan M, Hopkins A, Jaffee EM. Tumor mutational burden and response rate to PD-1 inhibition. *N Engl J Med* 2017;377: 2500–1.
- Powles T, Duran I, van der Heijden MS, Loriot Y, Vogelzang NJ, De Giorgi U, et al. Atezolizumab versus chemotherapy in patients with platinum-treated locally advanced or metastatic urothelial carcinoma (IMvigor211): a multicentre, open-label, phase 3 randomised controlled trial. *Lancet* 2018;391:748–57.
- Rittmeyer A, Barlesi F, Waterkamp D, Park K, Ciardiello F, von Pawel J, et al. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. *Lancet* 2017;389:255–65.
- Rosenberg JE, Hoffman-Censits J, Powles T, van der Heijden MS, Balar AV, Necchi A, et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. *Lancet* 2016;387:1909–20.
- Powles T, Eder JP, Fine GD, Braiteh FS, Loriot Y, Cruz C, et al. MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. *Nature* 2014;515:558–62.
- McGranahan N, Furness AJ, Rosenthal R, Ramskov S, Lyngaa R, Saini SK, et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. *Science* 2016;351:1463–9.
- Teng MW, Ngiew SF, Ribas A, Smyth MJ. Classifying cancers based on T-cell infiltration and PD-L1. *Cancer Res* 2015;75:2139–45.
- Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* 2014;515:568–71.
- Maleki Vareki S, Garrigos C, Duran I. Biomarkers of response to PD-1/PD-L1 inhibition. *Crit Rev Oncol Hematol* 2017;116:116–24.
- Hodi FS, Kluger HM, Sullivan RJ, Flaherty KT, Soria JC, Xiao Y, et al. Clinical activity of the PD-L1 inhibitor MPDL3280A in patients with metastatic melanoma: updated phase I data. *Soc Melanoma Res* 2014.
- Tecentriq (atezolizumab). [package insert]. South San Francisco, CA: Genentech, Inc.; 2019.
- Tecentriq (atezolizumab). [summary of product characteristics]. Welwyn Garden City, United Kingdom: Roche Registration Limited; 2018.
- Petrylak DP, Powles T, Bellmunt J, Braiteh F, Loriot Y, Morales-Barrera R, et al. Atezolizumab (MPDL3280A) monotherapy for patients with metastatic urothelial cancer: long-term outcomes from a phase 1 study. *JAMA Oncol* 2018;4:537–44.
- Balar AV, Galsky MD, Rosenberg JE, Powles T, Petrylak DP, Bellmunt J, et al. Atezolizumab as first-line treatment in cisplatin-ineligible patients with locally advanced and metastatic urothelial carcinoma: a single-arm, multicentre, phase 2 trial. *Lancet* 2017;389:67–76.
- Mariathasan S, Turley SJ, Nickles D, Wang Y, Kadel EE III, Koeppen H, et al. TGF- β attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. *Nature* 2018;554:544–8.

24. Frampton GM, Fichtenholtz A, Otto GA, Wang K, Downing SR, He J, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. *Nat Biotechnol* 2013;31:1023–31.
25. R Package Documentation. JasonHackney/GSDecon: deconvolution of cellular proportions in expression data; 2018. Available from: <https://rdrr.io/github/JasonHackney/GSDecon/>.
26. Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, et al. Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. *N Engl J Med* 2018;379:2108–21.
27. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* 2015;348:124–8.
28. Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N Engl J Med* 2014;371:2189–99.
29. Rosenberg J, Petrylak D, Van Der Heijden S, Necchi A, O'Donnell P, Loriot Y, et al. PD-L1 expression, cancer genome atlas (TCGA) subtype, and mutational load as independent predictors of response to atezolizumab (atezo) in metastatic urothelial carcinoma (mUC; IMvigor210). *J Clin Oncol* 34:15s,2016 (suppl; abstr 104).
30. Johnson DB, Frampton GM, Rieth MJ, Yusko E, Xu Y, Guo X, et al. Targeted next generation sequencing identifies markers of response to PD-1 blockade. *Cancer Immunol Res* 2016;4:959–67.
31. Spigel DR, Schrock AB, Fabrizio D, Frampton GM, Sun J, He J, et al. Total mutation burden (TMB) in lung cancer (LC) and relationship with response to PD-1/PD-L1 targeted therapies. *J Clin Oncol* 35:15s,2016 (suppl 15; abstr 9017).
32. Zaretsky JM, Garcia-Diaz A, Shin DS, Escuin-Ordinas H, Hugo W, Hu-Lieskovan S, et al. Mutations associated with acquired resistance to PD-1 blockade in melanoma. *N Engl J Med* 2016;375:819–29.
33. Peng W, Chen JQ, Liu C, Malu S, Creasy C, Tetzlaff MT, et al. Loss of PTEN promotes resistance to T cell-mediated immunotherapy. *Cancer Discov* 2016;6:202–16.
34. McDermott DF, Sosman JA, Sznol M, Massard C, Gordon MS, Hamid O, et al. Atezolizumab, an anti-programmed death-ligand 1 antibody, in metastatic renal cell carcinoma: Long-term safety, clinical activity, and immune correlates from a phase Ia study. *J Clin Oncol* 2016;34:833–42.
35. Ribas A, Hamid O, Daud A, Hodi FS, Wolchok JD, Kefford R, et al. Association of pembrolizumab with tumor response and survival among patients with advanced melanoma. *JAMA* 2016;315:1600–9.
36. D'Angelo SP, Larkin J, Sosman JA, Lebbe C, Brady B, Neyns B, et al. Efficacy and safety of nivolumab alone or in combination with ipilimumab in patients with mucosal melanoma: a pooled analysis. *J Clin Oncol* 2017;35:226–35.
37. Hodi FS, Kluger H, Sznol M, Carvajal R, Lawrence D, Atkins M, et al. Durable, long-term survival in previously treated patients with advanced melanoma (MEL) who received nivolumab (NIVO) monotherapy in a phase I trial [abstract]. In: Proceedings of the AACR 107th Annual Meeting 2016; April 16–20, 2016; New Orleans, LA. Philadelphia (PA): AACR; 2016. Abstract nrCT001.
38. Hamid O, Robert C, Daud A, Hodi FS, Hwu WJ, Kefford R, et al. 5-year survival outcomes in patients (pts) with advanced melanoma treated with pembrolizumab (pembro) in KEYNOTE-001. *J Clin Oncol* 36:15s,2018 (suppl; abstr 9516).
39. Carbognin L, Pilotto S, Milella M, Vaccaro V, Brunelli M, Calio A, et al. Differential activity of nivolumab, pembrolizumab and MPDL3280A according to the tumor expression of programmed death-ligand-1 (PD-L1): sensitivity analysis of trials in melanoma, lung and genitourinary cancers. *PLoS One* 2015;10:e0130142.
40. Weide B, Martens A, Hassel JC, Berking C, Postow MA, Bisschop K, et al. Baseline biomarkers for outcome of melanoma patients treated with pembrolizumab. *Clin Cancer Res* 2016;22:5487–96.
41. Kroeger DR, Milne K, Nelson BH. Tumor-infiltrating plasma cells are associated with tertiary lymphoid structures, cytolytic T-cell responses, and superior prognosis in ovarian cancer. *Clin Cancer Res* 2016;22:3005–15.
42. Wirsing AM, Rikardsen OG, Steigen SE, Uhlin-Hansen L, Hadler-Olsen E. Characterisation and prognostic value of tertiary lymphoid structures in oral squamous cell carcinoma. *BMC Clin Pathol* 2014;14:38.
43. Lardone RD, Plaisier SB, Navarrete MS, Shamoni JM, Jallas JR, Sieling PA, et al. Cross-platform comparison of independent datasets identifies an immune signature associated with improved survival in metastatic melanoma. *Oncotarget* 2016;7:14415–28.
44. Garg K, Maurer M, Griss J, Bruggen MC, Wolf IH, Wagner C, et al. Tumor-associated B cells in cutaneous primary melanoma and improved clinical outcome. *Hum Pathol* 2016;54:157–64.
45. Hugo W, Zaretsky JM, Sun L, Song C, Moreno BH, Hu-Lieskovan S, et al. Genomic and transcriptomic features of response to anti-PD-1 therapy in metastatic melanoma. *Cell* 2016;165:35–44.
46. Sade-Feldman M, Jiao YJ, Chen JH, Rooney MS, Barzily-Rokni M, Eliane JP, et al. Resistance to checkpoint blockade therapy through inactivation of antigen presentation. *Nat Commun* 2017;8:1136.
47. McQuade JL, Daniel CR, Hess KR, Mak C, Wang DY, Rai RR, et al. Association of body-mass index and outcomes in patients with metastatic melanoma treated with targeted therapy, immunotherapy, or chemotherapy: a retrospective, multicohort analysis. *Lancet Oncol* 2018;19:310–22.
48. De Rosa V, La Cava A, Matarese G. Metabolic pressure and the breach of immunological self-tolerance. *Nat Immunol* 2017;18:1190–6.
49. Molinero LL, Yin D, Lei YM, Chen L, Wang Y, Chong AS, et al. High-fat diet-induced obesity enhances allograft rejection. *Transplantation* 2016;100:1015–21.