Impact of DNA Damage Response and Repair (DDR) Gene Mutations on Efficacy of PD-(L)1 Immune Checkpoint Inhibition in Non–Small Cell Lung Cancer

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ABSTRACT

Purpose: DNA damage response and repair (DDR) gene alterations are associated with increased tumor-infiltrating lymphocytes, higher genomic instability, and higher tumor mutational burden (TMB) in cancer. Whether DDR alterations are associated with clinical outcomes to programmed death ligand 1 [PD-(L)1] blockade in non–small cell lung cancer (NSCLC) is unknown.

Experimental Design: Tumors from patients treated with PD-(L)1 inhibitors were analyzed using targeted next-generation sequencing (NGS). Cancers were categorized on the basis of the presence or absence of deleterious mutations across a panel of 53 DDR genes. Clinical outcomes to PD-(L)1 inhibitors were evaluated according to DDR mutation status.

Results: Of 266 patients with successful NGS who received PD-(L)1 inhibitors, 132 (49.6%) were identified as having deleterious DDR mutations (DDR-positive). DDR-positive and DDR-negative groups were similar in terms of baseline clinicopathologic characteristics. The median TMB was significantly higher in the DDR-positive group compared with the DDR-negative group (12.1 vs. 7.6 mutations/megabase; $P < 0.001$). Compared with DDR-negative patients ($N = 134$), DDR-positive patients had a significantly higher objective response rate (30.3% vs. 17.2%; $P = 0.01$), longer median progression-free survival [PFS; 5.4 vs. 2.2 months; HR, 0.58 (95% confidence interval (CI), 0.45–0.76); $P < 0.001$], and longer median overall survival [OS; 18.8 vs. 9.9 months; HR, 0.57 (95% CI, 0.42–0.77); $P < 0.001$] with PD-(L)1 therapy. After adjusting for PD-L1, TMB, performance status, tobacco use, and line of therapy, DDR-positive status was associated with a significantly longer PFS [HR, 0.68 (95% CI, 0.51–0.92); $P = 0.01$] and OS [HR, 0.60 (95% CI, 0.43–0.85); $P = 0.004$] in multivariate analysis.

Conclusions: Deleterious DDR mutations are frequent in NSCLC and are associated with improved clinical outcomes in patients with NSCLC treated with PD-(L)1 blockade.

Introduction

Immune checkpoint blockade with programmed death 1 (PD-1) or programmed death ligand 1 (PD-L1) inhibitors is an integral component of standard treatment for most patients with advanced non–small cell lung cancer (NSCLC; refs. 1–7). However, the degree of benefit with PD-(L)1 inhibitor therapy is highly variable, and the identification of clinically available biomarkers of response to these agents in NSCLC has been challenging. Although PD-L1 expression levels by IHC broadly correlate to response to immunotherapy in NSCLC, patients with tumors across all PD-L1 expression levels (including negative expression) may derive prolonged clinical benefit from PD-(L)1 inhibitors, which highlights the need to identify novel biomarkers of immunotherapy efficacy.

Defects in a complex network of genes that mediate the cellular response to DNA damage have been associated with improved therapeutic sensitivity to platinum chemotherapy, PARP inhibitors, and other agents across multiple solid tumor types (8–12). Several PARP inhibitors have recently received FDA approval in ovarian, breast, and pancreatic cancers, primarily in patients harboring *BRCA* mutations (13–15). DNA repair deficiency is also an emerging biomarker of response to immune checkpoint blockade (10). Alterations in DNA damage response and repair (DDR) genes are associated with genomic instability and increased somatic tumor mutational burden (TMB), which may enhance immunogenicity through increased tumor-specific neoantigen load (10, 16–18). DDR gene alterations may also enhance immune recognition and targeting via neoantigen-independent pathways, including activation of innate antitumor immunity mediated by the stimulator of IFN genes (STING) pathway (19–22). The presence of DDR gene alterations was recently shown to be independently associated with clinical benefit to anti-PD-(L)1 checkpoint blockade in metastatic urothelial cancer (23).

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Translational Relevance

In this study, we demonstrate that pathogenic DNA damage response and repair (DDR) mutations are frequent in non-small cell lung cancer (NSCLC) and are associated with improved response rate, progression-free survival, and overall survival in patients with NSCLC treated with programmed death ligand 1 [PD-(L)1] inhibitor therapy. The identification and characterization of DDR mutation status in cancer has relevant implications for novel combinatorial immuno-oncology strategies. The combination of available predictive biomarkers of immunotherapy response such as PD-L1 expression with information on DDR mutation status may allow rational design of new combinatorial immunotherapy trials to enhance the proportion of patients with cancer who benefit from immunotherapy.

DDR gene alterations are common in NSCLC but are poorly characterized, and the clinical significance of these alterations remains unknown (24–29). We hypothesized that mutations in DDR genes are associated with higher TMB and improved clinical outcomes to PD-(L)1 inhibitor therapy in patients with advanced NSCLC.

Materials and Methods

Study design and patients

We collected clinicopathologic data from patients with advanced NSCLC who had consented to a correlative research study (DF/HCC protocol #02-180). Patients were included if they had received at least one dose of PD-(L)1 inhibitor alone or in combination with a CTLA-4 inhibitor. Patients receiving PD-(L)1 checkpoint blockade in combination with chemotherapy were excluded. All patients provided written consent to institutional review board–approved protocols at the Dana-Farber/Harvard Cancer Center (DF/HCC, Boston, MA) allowing for chart review and genomic sequencing on tissue samples (DF/HCC protocols #02-180). The study was conducted in accordance with the Declaration of Helsinki.

Targeted tumor next-generation sequencing

NSCLCs at the Dana-Farber Cancer Institute (DFCI, Boston, MA) were sequenced by targeted next-generation sequencing (NGS) using OncoPanel Version 3, which surveys exonic DNA sequences of 447 cancer genes, including 191 regions from 60 genes for rearrangement detection. DNA was isolated from tissue containing at least 20% tumor content and analyzed by massively parallel sequencing using a solution-phase Agilent SureSelect Hybrid Capture Kit and an Illumina HiSeq 2500 sequencer, as described previously (30). Common SNPs present in public and internal noncancer populations were filtered out algorithmically using an informatics pipeline (30). A total of 53 genes were classified as being related to DRR, which were grouped into functional pathways, based on literature review and expert curation (Supplementary Materials and Methods).

PD-L1 testing and TMB assessment

PD-L1 expression was reported as a percentage of tumor cells with positive membranous staining in a slide containing at least 100 tumor viable cells. TMB, defined as the number of somatic, coding, base substitution, and insertion/deletion (indel) mutations per megabase (Mb) of genome examined was calculated from the DFCI OncoPanel NGS platform as described previously (30).

Pathogenicity assessment and determination of deleterious mutation status

All loss-of-function mutations in DDR genes (including nonsense, frameshift, or splice site) were classified as deleterious (23). To determine the pathogenicity of missense mutations, we employed a three-step approach. First, we reviewed all the identified missense mutations in the Catalogue of Somatic Mutations in Cancer (COSMIC; ref. 31) and ClinVar (32) databases. Second, we performed an *in silico* functional analysis using the PolyPhen-2 (Polymorphism Phenotyping v2) prediction tool to determine the functional significance of each missense mutation (33). Third, because only tumor tissue was sequenced (without paired germline analysis), common SNPs were filtered if present at >0.1% frequency in Genome Aggregation Database (gnomAD) version 2.1.1 (http://gnomad.broadinstitute.org/). Missense mutations reported as pathogenic by COSMIC and/or ClinVar or with a PolyPhen-2 score of ≥0.95 ("probably damaging"), were classified as deleterious. Patients harboring one or more deleterious DDR mutations were defined as DDR positive, while patients without deleterious DDR mutations were defined as DDR negative.

Clinical outcomes

To determine objective response rate (ORR) and progression-free survival (PFS), scans were reviewed by a dedicated thoracic radiologist using RECIST version 1.1 (34). PFS was defined as the time from the start of PD-(L)1 inhibitor therapy to the date of disease progression or death, whichever occurred first. Patients who were alive without disease progression were censored on the date of their last adequate disease assessment. Overall survival (OS) was defined as the time from the start of PD-(L)1 inhibitor therapy to death. Patients who were still alive were censored at the date of last contact.

Statistical analysis

Categorical and continuous variables were summarized using descriptive statistics. The Wilcoxon rank sum test and Kruskal–Wallis test were used to test for differences between continuous variables, and Fisher exact test was used to test for associations between categorical variables. Event-time distributions were estimated using the Kaplan–Meier methodology, and log-rank tests were used to test for differences in event-time distributions. Cox proportional hazards regression models were used to obtain estimates of HRs in univariate and multivariate analysis. A variance inflation factor (VIF) was used to detect multicollinearity in regression analysis. All P values were two-sided and confidence intervals (CI) were at the 95% level, with statistical significance defined as $P \leq 0.05$. All statistical analyses were performed using R version 3.6.1 (The R Foundation for Statistical Computing).

Results

Patient characteristics

A total of 266 patients with advanced NSCLC and successful tumor NGS who were treated with PD-(L)1 inhibitor therapy at the DFCI (Boston, MA) between January 2014 and September 2018 were identified. The median age of the cohort was 66 years (range, 35–92), most patients had a history of tobacco use (83.5%), and the majority of tumors demonstrated adenocarcinoma histology (80.8%). In the entire cohort, an activating *KRAS* mutation was found in 33.4% of cases, while an *EGFR*-activating mutation was identified in 10.2% of cases. The median PD-L1 expression was 50% (interquartile range, 2.75–90), while the median TMB was 9.18 mutations/Mb (mut/Mb;

range, 0.76–54.75). The baseline clinical and pathologic characteristics of the 266 patients are detailed in Supplementary Table S1.

DDR mutation status and baseline characteristics

Tumors from 132 patients (49.6%) were defined as DDR positive, while the remaining 134 (50.4%) were defined as DDR negative (**Fig. 1**). Overall, 201 deleterious mutations in DDR genes were noted among the 132 DDR-positive cases (Supplementary Table S2). Of these, 143 of 201 (71.1%) consisted of missense mutations, while the remaining included nonsense, splice site, and frameshift alterations. Among DDR-positive NSCLCs, the most commonly mutated DDR genes were *ATM* (9.4%), *ATR* (4.8%), *BRCA2* (4.1%), *POLQ* (3.7%), and *RAD50* (3.0%; Supplementary Fig. S1). Fifteen tumors (11.3%) with deleterious DDR mutations in *RAD50* ($n = 2$), *BRCA2* ($n = 2$), *ATM, ATR, MLH3, NEIL1, BAP1, CHEK2, ERCC5, POLQ, RAD21, RAD51D*, and *XRCC4* ($n = 1$ each) also demonstrated concomitant copy loss in the respective gene, consistent with LOH. The baseline clinical and pathologic characteristics of the DDR-positive and DDR-negative groups were well balanced in terms of age, sex, performance status, histology, presence of brain metastasis prior to PD-(L)1 inhibitor treatment start, line of therapy, and PD-L1 expression level (**Table 1**).

The median TMB was significantly higher in the DDR-positive group compared with the DDR-negative group (12.1 vs. 7.6 mut/Mb; $P < 0.001$; **Fig. 2A**). Most patients had deleterious mutations in only one DDR gene (85/132, 64.4%), while 35.6% (47/132) of patients had mutations in ≥2 DDR genes. The median TMB was significantly higher among patients with ≥2 DDR gene mutations compared with those with one DDR gene mutation or with a DDR-negative genotype (15.2 vs. 10.6 vs. 7.6 mut/Mb; $P < 0.001$; **Fig. 2B**). Among smokers, DDR-positive cases had a significantly higher median TMB compared with DDR-negative cases (12.9 vs. 8.3 mut/Mb; $P < 0.001$; **Fig. 2C**). Similarly, among never smokers, DDR-positive cases had also a significantly higher median TMB compared with DDR-negative cases (8.7 vs. 5.7 mut/Mb; $P = 0.04$; **Fig. 2C**).

Association between DDR mutation status and clinical outcomes to PD-(L)1 checkpoint blockade

We next examined the clinical outcomes to PD-(L)1 inhibition according to DDR mutation status. In the entire cohort of 266 patients treated with PD-(L)1 inhibitor therapy, the ORR was 23.7% (95% CI, 18.7–29.2). At a median follow-up of 23.3 months (95% CI, 21.1–25.6), the median PFS (mPFS) was 3.3 months (95% CI, 2.5–4.1) and the median OS (mOS) was 13.8 months (95% CI, 11.8–17.2) calculated from the date of PD-(L)1 inhibitor initiation (Supplementary Fig. S2). In the DDR-positive group, the ORR was 30.3% (95% CI, 22.6–38.9), which was significantly higher compared with the ORR of 17.2% (95% CI, 11.2–24.6) observed in the DDR-negative group ($P = 0.01$; **Fig. 3A**). For responders, the median duration of response was 19.3 months [95% CI, 13.9–not reached (NR)] in the DDR-positive group and 10.8 months (95%CI, 7.9–NR) in the DDR-negative group (Supplementary Fig. S3). Among responders, 64.8% and 47.6% of DDR-positive patients had an ongoing response at 12 and 24 months, respectively, compared with the 46.7% and 19.5% of DDR-negative patients. The mPFS was significantly longer in the DDR-positive group compared with the DDR-negative group [5.4 vs. 2.2 months; HR, 0.58 (95% CI, 0.45–0.76); $P < 0.001$; **Fig. 3B**]. The mOS was also significantly longer in the DDR-positive group compared with the DDR-negative group [18.8 vs. 9.9 months; HR, 0.57 (95% CI, 0.42–0.77); $P < 0.001$; **Fig. 3C**]. As multicollinearity was not

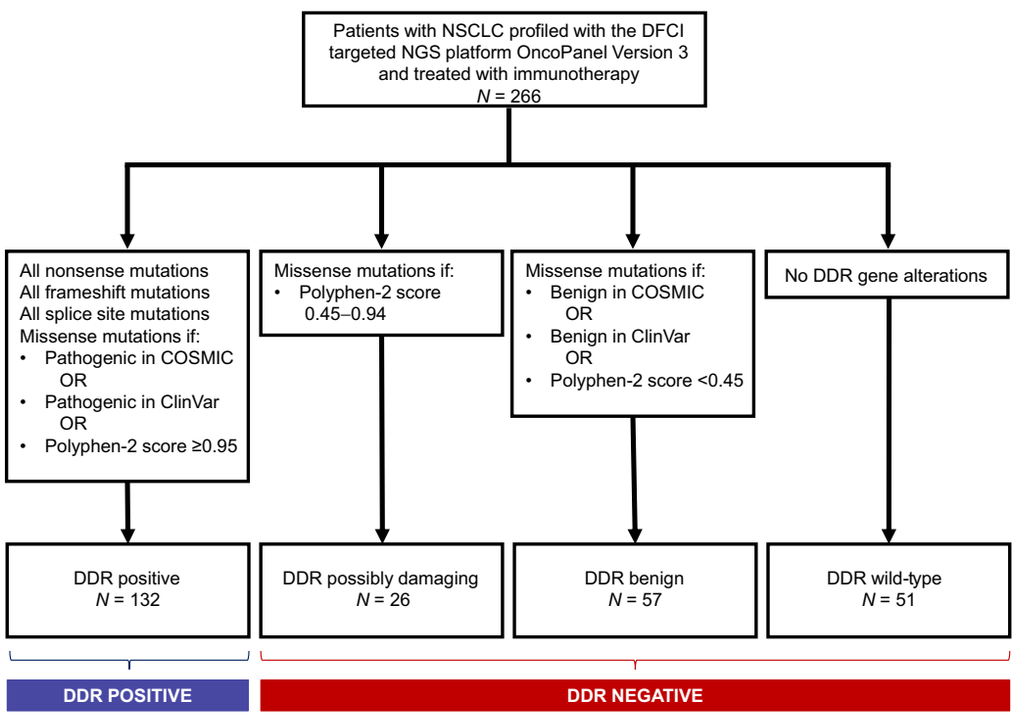

Figure 1.
Study flow chart of the 266 patients included in this study. A total of 132 NSCLCs (49.6%) were defined as DDR positive, while the remaining 134 (50.4%) were defined as DDR negative.

Table 1. Characteristics of patients with NSCLC by DDR mutation status.

Clinical characteristic	DDR positive *N* = 132 (%)	DDR negative *N* = 134 (%)	*P*
Age, median (range)	66 (35–92)	67 (35–90)	0.31
Sex			
Male	58 (43.9)	61 (45.5)	0.81
Female	74 (56.1)	73 (54.5)	
Smoking status			
Current/former	110 (83.3)	112 (83.6)	1.0
Never	22 (16.7)	22 (16.4)	
Histology			
Adenocarcinoma	106 (80.3)	109 (81.3)	0.59
Squamous cell carcinoma	19 (14.4)	15 (11.2)	
NSCLC NOS	7 (5.3)	10 (7.5)	
Oncogenic driver mutation			
KRAS	45 (34.1)	44 (32.8)	0.35
EGFR	9 (6.8)	18 (13.4)	
Other	22 (16.7)	20 (14.9)	
None identified	56 (42.4)	52 (38.8)	
Concurrent TP53 mutation			
Yes	78 (59.1)	86 (64.1)	0.45
No	54 (18.1)	48 (35.9)	
Concurrent STK11 mutation			
Yes	20 (15.2)	20 (14.9)	0.99
No	112 (84.8)	114 (85.1)	
ECOG performance status			
0–1	105 (79.5)	100 (76.6)	0.38
≥2	27 (20.5)	34 (25.4)	
Brain metastases prior to immunotherapy			
Yes	42 (31.8)	39 (29.1)	0.69
No	90 (68.2)	95 (70.9)	
Line of therapy			
1st	64 (48.5)	52 (38.8)	0.14
≥2nd	68 (51.5)	82 (61.2)	
PD-L1 expression			
<1%	12 (10.1)	22 (17.9)	0.21
1%–49%	41 (34.5)	37 (30.1)	
≥50%	66 (55.5)	64 (52.0)	
Not assessed	13	11	

Abbreviations: ECOG, Eastern Cooperative Oncology Group; NOS, not otherwise specified.

detected between PD-L1, TMB, DDR mutation status, and tobacco exposure with regard to clinical outcomes (VIFs < 3), all these variables were each included in the multivariate model. After adjusting for PD-L1 expression, TMB, performance status, line of therapy, and smoking status, the presence of a deleterious DDR mutation was associated with significantly longer PFS [HR, 0.68 (95% CI, 0.51–0.92); $P = 0.01$] and OS [HR, 0.60 (95% CI, 0.43–0.85); $P = 0.004$] in multivariate analysis (Supplementary Table S3). The unadjusted HRs for PFS and OS for each DDR genes pathway are shown in Supplementary Fig. S4.

Because a fraction of missense mutations included in our analysis were not included in COSMIC and/or ClinVar but predicted to be deleterious through PolyPhen-2 ($N = 108/143$, 75.6%), we also analyzed the clinical outcomes to immunotherapy according to the strength of evidence for DDR mutation status. We found that clinical outcomes to immunotherapy were similarly improved in both DDR-positive NSCLCs with loss-of-function or known pathogenic missense mutations as annotated by COSMIC and/or ClinVar ("DDR pathogenic", $N = 53$) and those with deleterious missense mutations as designated by PolyPhen-2 alone ("DDR predicted only", $N = 79$) when compared with the DDR-negative group, suggesting that DDR missense mutations defined as deleterious based on PolyPhen-2 alone are also associated with benefit from PD-(L)1 inhibitor therapy (Supplementary Fig. S5).

As first-line pembrolizumab represents a first-line treatment option for patients with NSCLC and a PD-L1 expression level of ≥50%, we also investigated the impact of DDR mutation status in this specific clinical context. In the entire cohort of 266 patients, 92 (34.6%) had NSCLC with a PD-L1 expression level of ≥50% and received first-line pembrolizumab monotherapy. In this group, 49 (53.3%) cases were DDR positive and 43 (46.7%) were DDR negative. Baseline clinical and pathologic features were well balanced between the two cohorts with the only exception of median TMB, which was significantly higher in the DDR-positive group compared with the DDR-negative group (13.7 vs. 7.6 mut/Mb; $P < 0.001$; Supplementary Table S4). The ORR was significantly higher in the DDR-positive group compared with the DDR-negative group [53.1% (95% CI, 38.2–67.5) vs. 25.6% (95% CI, 13.5–41.2); $P = 0.01$; **Fig. 4A**]. The mPFS was significantly longer in the DDR-positive group compared with the DDR-negative group [13.0 vs. 3.1 months; HR, 0.35 (95% CI, 0.21–0.60);

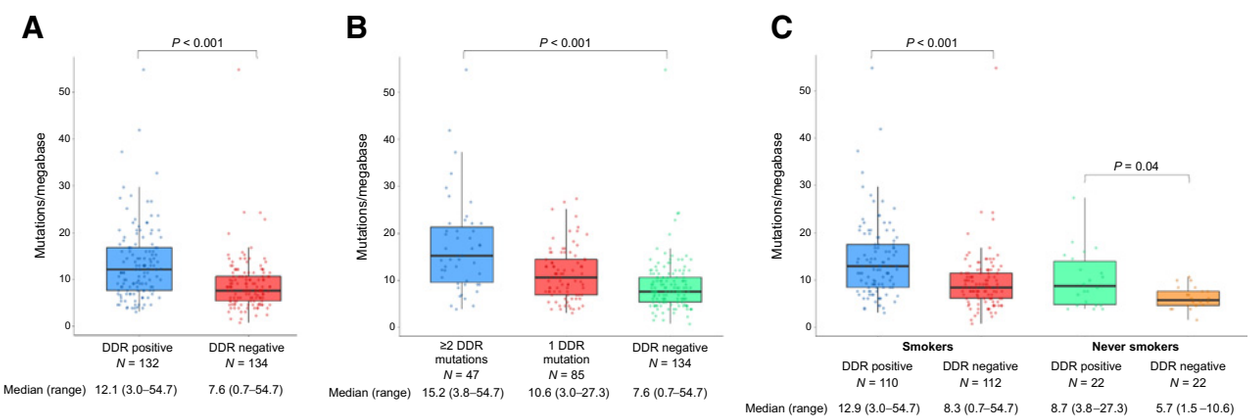

Figure 2.
A, TMB by DDR gene mutation status. **B,** TMB by the number of DDR gene alterations. **C,** TMB by DDR mutation among smokers and never smokers.

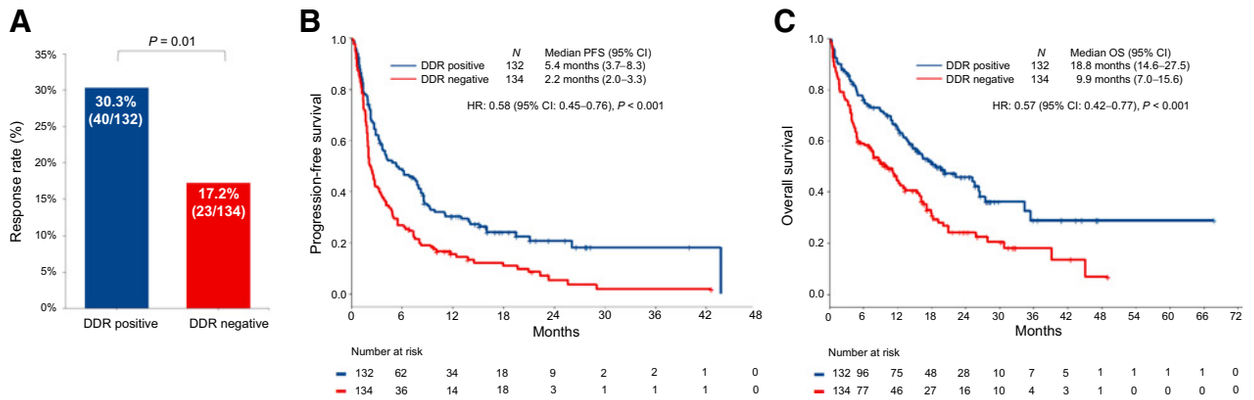

Figure 3.
Response rate (**A**), PFS (**B**), and OS (**C**) in patients treated with PD-(L)1 inhibitor therapy in the DDR-positive and DDR-negative groups.

$P < 0.001$; **Fig. 4B**]. The mOS was also significantly longer in the DDR-positive group compared with the DDR-negative group [NR vs. 13.3 months; HR, 0.37 (95% CI, 0.20–0.70); $P < 0.01$; **Fig. 4C**]. After adjusting for TMB and performance status, the presence of a deleterious DDR gene mutation was associated with significantly longer PFS [HR, 0.43 (95% CI, 0.24–0.78); $P = 0.01$] and OS [HR, 0.42 (95% CI, 0.21–0.86); $P = 0.02$] in multivariate analysis also among patients with a PD-L1 expression of ≥50% treated with first-line pembrolizumab monotherapy (Supplementary Table S5). To confirm that DDR mutations are associated with immunotherapy response, we also examined the relationship between DDR mutation status and clinical outcomes to first-line platinum doublet chemotherapy. Among the 266 patients treated with PD-(L)1 inhibitor therapy, 95 (35.7%), received a platinum doublet chemotherapy as first-line treatment. We found no difference in ORR (46.5% vs. 44.2%; $P = 0.83$; Supplementary Fig. S6A) or mPFS [4.9 vs. 5.2 months; HR, 0.98 (95% CI, 0.65–1.48); $P = 0.93$; Supplementary Fig. S6B] between the DDR-positive and the DDR-negative cohorts.

We next investigated the impact of the number of DDR mutations on the clinical outcome to immunotherapy and found that the presence of multiple DDR gene mutations was significantly associated with increased ORR to immunotherapy, with ORRs of 36.2% (95% CI, 22.7–51.5), 27.1% (95% CI, 17.9–37.7), and 17.2% (95% CI, 11.2–24.6) among tumors with ≥2, one, or no deleterious DDR gene alterations ($P = 0.02$; Supplementary Fig. S7A), respectively. The mPFS was also longest among patients with ≥2 DDR gene mutations compared with those with one or no DDR gene mutations (mPFS, 7.9 vs. 4.3 vs. 2.2 months, log-rank P value across all groups <0.001; Supplementary Fig. S7B). The mOS was significantly different across the three groups (16.5 vs. 19.8 vs. 9.9 months, log-rank P value across all groups = 0.001, respectively; Supplementary Fig. S7C).

We finally analyzed the clinical outcomes to PD-(L)1 inhibitor therapy among the DDR-positive and DDR-negative groups by smoking history. Among DDR-positive cases, the ORR was 31.8% (35/110) among current/former smokers and 22.7% (5/22) among never smokers. Among DDR-negative cases, the ORR was 17.9% (20/112) in current/former smokers and 13.6% (3/22) in never smokers (Supplementary Fig. S8A). The mPFS and the mOS significantly differed among the four groups and were longest in the DDR-positive/smoker group and shortest in the DDR-negative/never smoker group (log-rank P value across all groups = <0.001 and 0.003, respectively, for mPFS and mOS; Supplementary Fig. S8B and S8C).

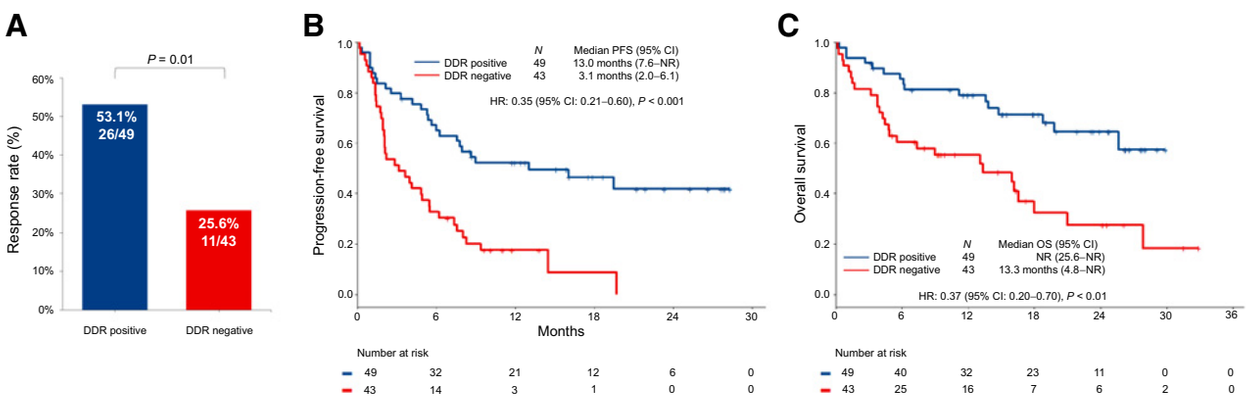

Figure 4.
Response rate (**A**), PFS (**B**), and OS (**C**) in patients with PD-L1 expression ≥50% treated with first-line pembrolizumab monotherapy in DDR-positive and DDR-negative NSCLC.

Discussion

In this study, we demonstrate that deleterious DDR mutations are common in advanced NSCLC, and the presence of these mutations is associated with improved clinical outcomes to treatment with PD-(L)1 inhibitors. We also demonstrate that this association is observed among patients with PD-L1 expression ≥50% treated with first-line pembrolizumab. Importantly, we found no difference in ORR and mPFS to first-line chemotherapy according to DDR mutation status.

To our knowledge, this is the first study to demonstrate an independent association between deleterious DDR gene mutations and clinical benefit to PD-(L)1 inhibitor therapy in patients with advanced NSCLC. PD-L1 expression by IHC is an imperfect predictive biomarker of PD-(L)1 inhibitor response, and the recent FDA approval of pembrolizumab monotherapy for patients with PD-L1 expression ≥1% (35) highlights an important and timely need for clinical tools that can distinguish patients who will benefit from PD-(L)1 inhibitor therapy alone versus those whose optimal treatment may be the combination of a PD-(L)1 inhibitor plus doublet chemotherapy. The increased utilization of broad genomic profiling in the contemporary care of advanced NSCLC suggests that DDR mutation status may be a readily available genomic biomarker that could augment treatment decision making.

Along with PD-L1 expression, higher nonsynonymous TMB is also associated with improved clinical outcomes to PD-1 blockade in patients with advanced NSCLC (36, 37). In our analysis, TMB was associated with a longer PFS to immunotherapy but not a prolonged OS in multivariate analysis, which is consistent with recent data showing an improvement in PFS but not in OS in patients with high TMB treated with PD-(L)1 inhibitor therapy (38, 39). Conversely, DDR mutation status was independently associated with improved PFS and OS in multivariate models, after controlling for TMB and PD-L1 expression. However, due to the collinearity between DDR mutations and TMB, the mutual independence of these two variables cannot be entirely demonstrated.

While these findings appear to be a class effect, this study was not powered for subset analyses of individual DDR genes. In addition to higher TMB and higher predicted neoantigenic load, other non-neoantigen–based mechanisms may contribute to this association. For example, activation of the STING pathway as a result of cytosolic DNA fragment accumulation in the setting of DDR deficiency is an emerging potential mechanism that can foster potent antitumor immune response (20, 39–43). Therefore, the presence of DDR mutation should not be interpreted simply as a proxy for higher TMB and neoantigen load. Rather, these two measures should be integrated with known predictive biomarkers, such as PD-L1 expression, to identify patients that are more likely to respond to PD-(L)1 inhibitor therapy. However, additional studies utilizing larger patients cohorts are needed to determine the impact of individual DDR genes or DDR functional classes on TMB, PD-L1 expression, and association with clinical outcome to PD-(L)1 blockade in NSCLC

The identification and characterization of DDR mutation status in NSCLC may also have implications for novel combinatorial immuno-oncology strategies. Clinical trials combining PD-(L)1 inhibition with DNA repair–targeted agents, including PARP and ATR inhibitors, in patients with DDR-mutant disease are ongoing. Combining PD-L1 expression levels with DDR mutation status might enable improved biomarker selection to enhance the proportion of patients with NSCLC who will benefit from PD-(L)1 inhibitors.

Our findings also highlight that pathogenicity assessment is an important challenge relevant to the interpretation of DDR gene mutations identified by clinical genomic profiling. We classified loss-of-function mutations in DDR genes (including nonsense, frameshift, or splice site) as deleterious, and integrated several tools (COSMIC, ClinVar, and PolyPhen-2) to determine the functional significance of missense mutations. When we analyzed the clinical outcomes to PD-(L)1 inhibitor therapy according to the strength of evidence for DDR mutation status, we found that clinical outcomes to immunotherapy were improved even for missense mutations that were not included in COSMIC and/or ClinVar but predicted to be deleterious through PolyPhen-2, compared with the DDR-negative cohort. Nonetheless, the number of missense mutations that were not included in COSMIC and/or ClinVar highlights that additional functional validation of DDR gene mutations in NSCLC is highly warranted.

We acknowledge several limitations relevant to this study: (i) this was a retrospective analysis of patients treated at a single academic cancer center; (ii) a fraction of the mutations identified by this analysis have not had robust functional characterization; (iii) COSMIC and ClinVar databases are dynamic, and the extent of functional validation underlying pathogenicity annotations in these databases is variable; (iv) dedicated paired germline analysis was not performed; and (v) OncoPanel is a targeted NGS assay that does not include coverage of all DDR genes (44).

In conclusion, our data demonstrate that deleterious DDR gene mutations in advanced NSCLC are associated with improved clinical outcomes to PD-(L)1 inhibitor therapy in NSCLC and may represent a novel biomarker for immunotherapy efficacy in NSCLC. Additional prospective studies with larger sample sizes are needed for the independent validation of these findings, and to permit more robust analysis of individual DDR genes or gene subsets. Further investigation into the mechanistic basis of this association represents an important priority.

Disclosure of Potential Conflicts of Interest

G. Recondo is an paid consultant for Roche, Amgen, and Pfizer. A.D. Cherniack is an paid consultant for LabCorp, reports receiving other commercial research support from Bayer AG, and holds ownership interest (including patents) in Merck. M. Nishino is an paid consultant for Daiichi Sankyo and AstraZeneca, and reports receiving commercial research grants (to the institution) from Canon Medical Systems and AstraZeneca. L.M. Sholl is an paid consultant for EMD Serono and reports receiving commercial research grants from Roche/Genentech. G.I. Shapiro is an paid consultant for Merck/EMD Serono, Artios, Bayer, Cybrexa Therapeutics, Sierra Oncology, and Ipsen, and reports receiving commercial research grants from Merck/EMD Serono, Merck & Co., Sierra Oncology, and Lilly Oncology. M.M. Awad is an paid consultant for Achilles, AbbVie, Neon, Maverick, Blueprint, Hengrui, Nektar, Syndax, Bristol-Myers Squibb, and AstraZeneca, and reports receiving commercial research grants from Bristol-Myers Squibb, Lilly, AstraZeneca, and Genentech. M.L. Cheng is an paid consultant for AstraZeneca and Inivata, and reports receiving other remuneration from PCME (supported by Lilly and Merck), The Lynx Group (supported by Bristol-Myers Squibb), WedMD (supported by AstraZeneca), Sanofi-Aventis, Allergan, Daiichi Sankyo, Natera, and Guardant. No potential conflicts of interest were disclosed by the other authors.

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