Title: 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

Running Title: DDR gene mutations and immunotherapy efficacy in NSCLC.

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Statement of translational relevance: In this study we demonstrate that pathogenic DDR mutations are frequent in NSCLC and are associated with improved response rate, progression-free survival and overall survival in patients with NSCLC treated with 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 cancer patients who benefit from immunotherapy.
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 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 based on 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 clinicopathological characteristics. The median TMB was significantly higher in the DDR-positive group compared to the DDR-negative group (12.1 vs 7.6 mutations/megabase, P<0.001). Compared to 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 (5.4 vs 2.2 months, HR: 0.58 [95%CI:0.45-0.76], P<0.001), and longer median overall survival (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, PS, 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.
Conclusion: Deleterious DDR mutations are frequent in NSCLC and are associated with improved clinical outcomes in patients with NSCLC treated with PD-(L)1 blockade.

Keywords: DNA damage response and repair, TMB, biomarkers, PD-(L)1 blockade, NSCLC
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) [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 immunohistochemistry 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, 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 interferon genes (STING) pathway [19-22].
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]. 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 tumor mutational burden (TMB) and improved clinical outcomes to PD-(L)1 inhibitor therapy in patients with advanced NSCLC.

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 1 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) 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

Non-small cell lung cancers at the Dana-Farber Cancer Institute (DFCI) were sequenced by targeted next-generation sequencing 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
previously described [30]. Common single nucleotide polymorphisms 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 DNA damage
response and repair, which were grouped into functional pathways, based on literature
review and expert curation (Supplementary Methods SM).

PD-L1 testing and tumor mutational burden 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 next-generation sequencing (NGS) platform as previously described [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) [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 single nucleotide polymorphisms (SNPs) were filtered if present at >0.1% frequency in Genome Aggregation Database (gnomAD) version 2.1.1 (http://gnomad.broadinstitute.org/ last accessed May 15th, 2019). 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 ORR and progression-free survival (PFS), scans were reviewed by a dedicated thoracic radiologist using Response Evaluation Criteria In Solid Tumors (RECIST) version 1.1 [34]. Progression-free survival (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’s 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 hazard ratios in univariate and multivariable analysis. A variance inflation factor (VIF) was used to detect multicollinearity in regression analysis. All p-values are two-sided and confidence intervals are 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 Dana-Farber Cancer Institute (DFCI) 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/Megabase (mut/Mb) (range: 0.76-54.75). The baseline clinical and
pathological 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 (Figure 1). Overall, 201 deleterious mutations in DDR genes were noted among the 132 DDR-positive cases (Supplementary Table S2). Of these, 143/201 (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 Figure 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*, *XRCC4* (n = 1 each) also demonstrated concomitant copy loss in the respective gene, consistent with loss of heterozygosity. The baseline clinical and pathological 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 to the DDR-negative group (12.1 versus 7.6 mut/Mb, P<0.001) (Figure 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 to those with one DDR gene mutation or with a DDR-negative genotype (15.2 versus 10.6 versus 7.6 mut/Mb, P<0.001, Figure 2B). Among smokers, DDR-positive cases had a significantly higher median TMB compared to DDR-negative cases (12.9 versus 8.3 mut/Mb, P<0.001, Figure 2C). Similarly, among never smokers, DDR-positive cases had also a significantly higher median TMB compared to DDR-negative cases (8.7 versus 5.7 mut/Mb, P=0.04, Figure 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 was 3.3 months (95%CI: 2.5-4.1) and the median OS was 13.8 months (95%CI: 11.8-17.2) calculated from the date of PD-(L)1 inhibitor initiation (Supplementary Figure S2). In the DDR-positive group, the ORR was 30.3% (95%CI: 22.6-38.9), which was significantly higher compared to the ORR of 17.2% (95%CI: 11.2-24.6) observed in the DDR-negative group (P = 0.01, Figure 3A). For responders, the median duration of response was 19.3 months (95%CI: 13.9-not reached) in the DDR-positive group and 10.8 months (95%CI: 7.9-not reached) in the DDR-negative group (Supplementary Figure S3). Among responders, 64.8% and 47.6% of DDR-positive patients had an ongoing response at 12 and 24 months, respectively, compared to the 46.7% and 19.5% of DDR negative patients. The median
PFS (mPFS) was significantly longer in the DDR-positive group compared to the DDR-negative group (5.4 versus 2.2 months, HR: 0.58 [95%CI: 0.45-0.76], P<0.001, Figure 3B). The median OS (mOS) was also significantly longer in the DDR-positive group compared to the DDR-negative group (18.8 versus 9.9 months, HR: 0.57 [95%CI: 0.42-0.77], P<0.001, Figure 3C). As multicollinearity was not detected between PD-L1, TMB, DDR mutation status, and tobacco exposure with regard to clinical outcomes (variance inflation factors < 3), all these variables were each included in the multivariable 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 hazard ratios (HRs) for PFS and OS for each DDR genes pathway are shown in Supplementary Figure 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 to 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 Figure 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 pathological 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 to the DDR-negative group (13.7 versus 7.6 mut/Mb, P<0.001) (Supplementary Table S4). The ORR was significantly higher in the DDR-positive group compared to the DDR-negative group (53.1% [95%CI: 38.2-67.5] versus 25.6% [95%CI: 13.5-41.2], P=0.01, Figure 4A). The mPFS was significantly longer in the DDR-positive group compared to the DDR-negative group (13.0 versus 3.1 months, HR: 0.35 [95%CI: [0.21-0.60], P<0.001, Figure 4B). The mOS was also significantly longer in the DDR-positive group compared to the DDR-negative group (not reached [NR] versus 13.3 months, HR: 0.37 [95%CI: 0.20-0.70], P<0.01, Figure 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 Figure S6A) or mPFS (4.9 vs 5.2 months, HR: 0.98 [95%CI: 0.65-1.48], $P = 0.93$, Supplementary Figure 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 Figure S7A), respectively. The mPFS was also longest among patients with ≥2 DDR gene mutations compared to those with one or no DDR gene mutations (mPFS: 7.9 versus 4.3 versus 2.2 months, log rank P value across all groups <0.001 Supplementary Figure S7B). The mOS was significantly different across the 3 groups (16.5 versus 19.8 versus 9.9 months, log rank P value across all groups =0.001, respectively, Supplementary Figure S7C).

We lastly 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 Figure
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 Figure S8B-C).

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 immunohistochemistry is an imperfect predictive biomarker of PD-(L)1 inhibitor response, and the recent United States Food and Drug Administration 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 tumor mutational burden 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 multivariable 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 tumor mutational burden and higher predicted neoantigenic load, other non-neoantigen based mechanisms may contribute to this association. For example, activation of the stimulator of interferon genes (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

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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 NSCLC patients who 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 to 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: 1) this was a retrospective analysis of patients treated at a single academic cancer center; 2) a
fraction of the mutations identified by this analysis have not had robust functional
classification characterization; 3) COSMIC and ClinVar databases are dynamic, and the extent of
functional validation underlying pathogenicity annotations in these databases is
variable; 4) dedicated paired germline analysis was not performed; 5) 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.

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(WCLC).

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doi:10.1093/nar/gkw1121


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**Figure Legends**

**Figure 1.** Study flow chart of the 266 patients included in this study. One hundred thirty-two NSCLCs (49.6%) were defined as DDR-positive, while the remaining 134 (50.4%) were defined as DDR-negative. DDR, DNA damage response and repair.

**Figure 2.** (A) Tumor mutational burden (TMB) by DDR gene mutation status. (B) Tumor mutational burden by the number of DDR gene alterations. (C) Tumor mutational
burden by DDR mutation among smokers and never smokers. DDR, DNA damage response and repair.

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

**Figure 4. (A)** Response rate, (B) progression-free and (C) overall survival in patients with PD-L1 expression ≥50% treated with first-line pembrolizumab monotherapy in DDR-positive and DDR-negative NSCLC.
Table 1: Characteristics of patients with NSCLC by DDR mutation status

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<th>DDR negative N = 134 (%)</th>
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<td>56 (42.4)</td>
<td>52 (38.8)</td>
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</tr>
<tr>
<td>Concurrent TP53 mutation</td>
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<td></td>
<td>0.45</td>
</tr>
<tr>
<td>Yes</td>
<td>78 (59.1)</td>
<td>86 (64.1)</td>
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</tr>
<tr>
<td>No</td>
<td>54 (18.1)</td>
<td>48 (35.9)</td>
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</tr>
<tr>
<td>Concurrent STK11 mutation</td>
<td></td>
<td></td>
<td>0.99</td>
</tr>
<tr>
<td>Yes</td>
<td>20 (15.2)</td>
<td>20 (14.9)</td>
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</tr>
<tr>
<td>No</td>
<td>112 (84.8)</td>
<td>114 (85.1)</td>
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<tr>
<td>ECOG performance status</td>
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<td>0.38</td>
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<tr>
<td>0-1</td>
<td>105 (79.5)</td>
<td>100 (76.6)</td>
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</tr>
<tr>
<td>≥2</td>
<td>27 (20.5)</td>
<td>34 (25.4)</td>
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<td>Brain metastases prior to immunotherapy</td>
<td></td>
<td></td>
<td>0.69</td>
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<tr>
<td>Yes</td>
<td>42 (31.8)</td>
<td>39 (29.1)</td>
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</tr>
<tr>
<td>No</td>
<td>90 (68.2)</td>
<td>95 (70.9)</td>
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<tr>
<td>Line of therapy</td>
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<td>0.14</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>64 (48.5)</td>
<td>52 (38.8)</td>
<td></td>
</tr>
<tr>
<td>≥2&lt;br&gt;2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>68 (51.5)</td>
<td>82 (61.2)</td>
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<td>PD-L1 expression</td>
<td></td>
<td></td>
<td>0.21</td>
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<tr>
<td>&lt;1%</td>
<td>12 (10.1)</td>
<td>22 (17.9)</td>
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<tr>
<td>1-49%</td>
<td>41 (34.5)</td>
<td>37 (30.1)</td>
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<tr>
<td>≥50%</td>
<td>66 (55.5)</td>
<td>64 (52.0)</td>
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</tr>
<tr>
<td>Not assessed</td>
<td>13</td>
<td>11</td>
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</table>

ECOG, Eastern Cooperative Oncology Group
NSCLC NOS, non-small cell lung cancer not otherwise specified
PD-L1, programmed death ligand 1

27
Patients with NSCLC profiled with the DFCI targeted NGS platform OncoPanel Version 3 and treated with immunotherapy
N = 266

- All nonsense mutations
- All frameshift mutations
- All splice site mutations

Missense mutations if:
- Polyphen-2 score 0.45 - 0.94
- Pathogenic in COSMIC
- Pathogenic in ClinVar
- Polyphen-2 score ≥0.95

Missense mutations if:
- Polyphen-2 score <0.45
- Benign in COSMIC
- Benign in ClinVar

No DDR gene alterations

DDR Positive
N = 132

DDR Possibly Damaging
N = 26

DDR Benign
N = 57

DDR Wild Type
N = 51
Fig. 2

A

B

C

Authors have been notified of the acceptance of this manuscript but have not yet been edited. Author Manuscript Published OnlineFirst on April 24, 2020; DOI: 10.1158/1078-0432.CCR-19-3529
**Fig. 3**

**A**
- Response rate (%)
  - DDR positive: 30.3% (40/132)
  - DDR negative: 17.2% (23/134)
  - P = 0.01

**B**
- Progression-free survival
  - DDR positive: Median PFS 5.4 months (95% CI: 3.7-8.3)
  - DDR negative: Median PFS 2.2 months (95% CI: 2.0-3.3)
  - HR: 0.57 [95%CI: 0.42-0.77], P < 0.001

**C**
- Overall survival
  - DDR positive: Median OS 18.8 months (95% CI: 14.6-27.5)
  - DDR negative: Median OS 9.9 months (95% CI: 7.0-15.6)
  - HR: 0.57 [95%CI: 0.42-0.77], P < 0.001
<table>
<thead>
<tr>
<th>DDR status</th>
<th>N</th>
<th>Median OS (95% CI)</th>
<th>HR, 95% CI</th>
<th>P-value</th>
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<tbody>
<tr>
<td>DDR positive</td>
<td>49</td>
<td>NR (25.6-NR)</td>
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<td></td>
</tr>
<tr>
<td>DDR negative</td>
<td>43</td>
<td>13.3 months (4.8-NR)</td>
<td>0.35 [0.21-0.70]</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

**Fig. 4**

A) **Response rate (%):**

- DDR positive: 53.1% (26/49)
- DDR negative: 25.6% (11/43)

B) **Progression-free survival:**

- DDR positive: Median PFS = 13.0 months (7.6-NR)
- DDR negative: Median PFS = 3.1 months (2.0-6.1)

HR: 0.37 [95%CI: 0.20-0.70], P < 0.01

C) **Overall survival:**

- DDR positive: Median OS = NR (25.6-NR)
- DDR negative: Median OS = 13.3 months (4.8-NR)

HR: 0.37 [95%CI: 0.20-0.70], P < 0.01
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

Biagio Ricciuti, Gonzalo Recondo, Liam F Spurr, et al.

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