Original Article

DNA damage repair gene mutations predict the efficacy of platinum-based chemotherapy and immunotherapy plus platinum-based chemotherapy in advanced non-small cell lung cancer: a retrospective Chinese cohort study

Zhiwei Xiao[1#], Lingling Sun[1#], Yating Zheng[2], Hanrui Chen[1], Xinting Zheng[1], Jiamin Luo[1], Chuying Gu[1], Ruiting Lin[3], Mengli Huang[2], Yuezong Bai[2], Zhe-Sheng Chen[4], Connor J. Kinslow[5], Jerold Loh[6], Lizhu Lin[1]

[1]Oncology Center, The First Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou, China; [2]The Medical Department, 3D Medicines Inc., Shanghai, China; [3]The First Clinical Medical College of Guangzhou University of Chinese Medicine, Guangzhou, China; [4]Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences, St. John's University, Queens, NY, USA; [5]Department of Radiation Oncology, Vagelos College of Physicians and Surgeons, Columbia University Irving Medical Center, New York, NY, USA; [6]Department of Haematology-Oncology, National University Cancer Institute, Singapore (NCIS), National University Health System, Singapore, Singapore

Contributions: (I) Conception and design: Z Xiao, L Sun; (II) Administrative support: L Lin; (III) Provision of study materials or patients: L Lin, ZS Chen, Y Zheng, M Huang, Y Bai; (IV) Collection and assembly of data: H Chen, X Zheng, J Luo, C Gu, R Lin; (V) Data analysis and interpretation: Z Xiao, L Sun, Y Zheng; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work and should be considered as co-first authors.

Correspondence to: Lizhu Lin. Oncology Center, The First Affiliated Hospital of Guangzhou University of Chinese Medicine, 16 Airport Road, Guangzhou 510405, China. Email: lizhulin26@yahoo.com.

Background: Platinum-based chemotherapy (PC) and immunotherapy plus platinum-based chemotherapy (IPC) remain the first-line treatment for advanced NSCLC. But only a minority patients benefit from PC, and existing biomarkers, such as PD-L1, have been shown to be defective in predicting the efficacy of IPC. Highlighting the need to identify novel biomarkers for the efficacy of PC and IPC. DNA damage repair (DDR) mutations are known to predict response to PC in solid tumors. However, the predictive value of DDR in PC and IPC of NSCLC remains unclear.

Methods: Patients diagnosed with advanced or metastatic NSCLC were retrospectively included if they underwent next generation sequencing prior to starting treatment. Primary endpoints were to explore whether DDR mutations (DDRmut) are associated with clinical outcomes of PC and IPC. Secondary end point were to explore the association between DDRmut and the choice to add immunotherapy to chemotherapy, and the impact of different DDR pathways on efficacy in PC and IPC.

Results: DDRmut showed a strong association with tumor mutation burden-high (TMB-H) versus DDR wild-type (DDRwt) and higher rates of PD-L1 TPS ≥50% positivity. In 63 patients treated with PC, ORRs were 15.38% and 2.86% for DDRmut and DDRwt subgroup (P=0.1536), and DCRs were 88.46% and 45.72% (P=0.00097) at 6 months after PC. The DDRmut patients had significantly improved median PFS (mPFS) and median overall survival (mOS) than DDRwt group (mPFS: 7.6 *vs.* 3.9 months, HR =1.93, 95% CI: 1.09 to 3.14, P=0.0220. mOS: 29.9 *vs.* 20.7 months, HR =2.31, 95% CI: 1.09 to 4.9, P=0.0250). Moreover, among 37 patients treated with IPC, ORRs were 45% and 11.76% for DDRmut and DDRwt patients (P=0.0365), and the DCRs were 95% and 70.58% (P=0.0752), respectively at 6 months after IPC. The DDRmut patients had significantly improved mPFS compared to the DDRwt group (19.5 *vs.* 4.5 months, HR =3.28, 95% CI: 1.53 to 9.56, P=0.0022). In DDRmut group, mPFS of IPC recipients was significantly better than that of PC recipients (19.5 *vs.* 7.6 months, HR =2.09, 95% CI: 0.98 to 4.42, P=0.050).

Conclusions: There is potential for DDR to serve as a positive predictor of PC and IPC in advanced NSCLC patients.

Keywords: Non-small cell lung cancer (NSCLC); platinum-based chemotherapy (PC); immunotherapy plus platinum-based chemotherapy (IPC); DNA damage repair (DDR); prediction

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Introduction

The non-small cell lung cancer (NSCLC) treatment landscape has been evolving rapidly over the past decade, with surgery, targeted therapies, immune checkpoint inhibitors (ICIs), chemotherapy, and radiation therapy as key components of disease management (1). For patients with advanced stage NSCLC, since the discovery of epidermal growth factor receptor (*EGFR*), driver genes including anaplastic lymphoma kinase (*ALK*) and ROS proto-oncogene 1 (*ROS1*) have been discovered successively, and targeted therapy has heralded a new era (2-4). Patients receiving targeted therapy have exhibited a significantly improved median overall survival (mOS) compared to those who did not receive targeted therapy, making it the preferred therapy for patients with advanced NSCLC who are driver gene-positive (5). Subsequently, immunotherapy became another milestone alongside targeted therapy (6). Undoubtedly, NSCLC is one of the cancer types to benefit the most from immunotherapy due to the use of programmed cell death 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) ICIs. However, multiple studies have shown that ICI monotherapy has excellent performance only in patients with high programmed cell death-ligand 1 (PD-L1) expression (7-9). Therefore, in driver-gene negative NSCLC with negative or low PD-L1 expression, immunotherapy plus platinum-based chemotherapy (henceforth "IPC") is the most commonly used guideline-directed treatment, additionally, IPC is recommended even for patients with high PD-L1 levels, and immunotherapy monotherapy is recommended for those without crisis (10).

Although the degree of benefit of IPC differs based PD-L1 levels, current studies do not support the use of PD-L1 as a prognostic marker to identify patients who would not benefit from IPC. In KEYNOTE-189, in the comparison of PD-L1 TPS ≥50%, PD-L1 TPS 1–49% and PD-L1 TPS <1% subgroups, the mOS was not reached versus 21.8 months versus 17.8 months, respectively (11). Thus, it may be necessary to search for more predictive biomarkers to identify responders and non-responders with IPC. In addition, despite the rapid development of targeted therapies, the standard of care for a significant proportion of patients with driver negative is immunotherapy (or dual immunotherapy) and chemotherapy (12-15). Platinum-based chemotherapy (henceforth "PC") is still the standard chemotherapy backbone for advanced NSCLC. In the ECOG1594 study, PC has been associated with a 19% objective response rate (ORR), a median progression-free survival (mPFS) of 3.6 months and a median overall survival (mOS) of 7.9 months in NSCLC (regardless of pathologic type or smoking status, etc.) (16). It is clear not every patient benefit to the same degree, and further delineation to decide on upfront treatment strategy will be helpful. For example, in the IPASS study, the mOS of non-smoking lung adenocarcinoma patients treated with carboplatin plus paclitaxel was 17.4 months (17). However, there is still a lack of biomarkers that can better distinguish the corresponding patients with survival benefits, and no studies

Highlight box

Key findings

- DDR genes may be a positive predictor of platinum-based chemotherapy, and immunotherapy plus platinum-based chemotherapy in patients with advanced NSCLC.

What is known and what is new?

- DDR mutations are known to predict response to platinum-based chemotherapy in some solid tumors.
- The predictive value of DDR mutations in platinum-based chemotherapy, and immunotherapy plus platinum-based chemotherapy of NSCLC. For patients with DDR mutations, median PFS receiving immunotherapy plus platinum-based chemotherapy was significantly better than those receiving platinum-based chemotherapy.

What is the implication, and what should change now?

- These results suggest that patients with DDR mutations can receive either platinum-based chemotherapy or immunotherapy plus platinum-based chemotherapy better than those with DDR wild-type, and immunotherapy plus platinum-based chemotherapy can be recommended preferentially.

have shown which groups of people may benefit the most from PC.

Overall, despite the increasing use of targeted therapies, NSCLC patients with driver gene-negative still do not benefit from these therapies. PC and IPC remain the standard first-line treatment for advanced NSCLC. However, PC has a high toxicity rate, only a small number of patients benefit from PC, there are no effective biomarkers predicting the efficacy of PC. Although immunohistochemical detection of PD-L1 expression level and the immune response to treatment of NSCLC related extensively, but the existing studies (10,11) have shown that has all PD-L1 expression level (including negative expression) of cancer patients are likely to gain long-term clinical immunotherapy, highlighting the recognition immune treatment curative effect of the necessity of new biomarkers. In addition, for patients who are unable to receive targeted therapy, biomarkers should be explored to indicate whether a patient is a better candidate for PC or IPC.

Platinum compounds exert their cytotoxic effects by forming platinum-DNA adducts that interfere with DNA repair and inhibit transcription (18). DNA-repair capacity is considered both a barrier to tumorigenesis and a crucial molecular pathway involved in resistance to PC (19). In addition, DNA damage repair (DDR) is also an emerging biomarker for immunotherapy (20). DDR gene mutations are associated with genomic instability and increased somatic tumor mutational burden (TMB), which may enhance immunogenicity by increasing tumor-specific neoantigen burden (20-23). DDR gene mutations may also enhance immune recognition and targeting through neoantigen-independent pathways (23-27). Some studies have suggested that DNA-repair capacity is both a barrier to tumorigenesis and a crucial molecular pathway involved in resistance to PC and immunotherapy (19). Inactivation of mutations of genes in DDR pathways are frequently observed in cancer. According to previous literature, there are eight DDR pathways: mismatch repair (MMR), base excision repair (BER), damage sensor (DS), Fanconi anemia (FA), homologous recombination repair (HRR), nucleotide excision repair (NER), non-homologous end-joining (NHEJ), and DNA translesion synthesis (TLS) (28). The presence of DDR mutations has been reported to correlate with improved clinical outcomes in urothelial carcinoma (29), breast cancer (30), and prostate cancer (31). Previous studies have shown that downregulation of proteins of DDR pathways is associated with worse prognosis of stage I NSCLC patients having undergone surgery, as well as with increased efficacy of PC in locally advanced or metastatic NSCLC patients (32-34), but the correlation between DDR gene mutation and platinum chemotherapy efficacy in advanced NSCLC has not been verified in clinical studies. Similarly, although previous research has shown that DDR gene mutations are associated with immunotherapy efficacy in NSCLC (35), its correlation with IPC has not been reported. In addition, with the widely application of next generation sequencing (NGS), which can detect multiple genes at the same time, studies have shown that deleterious or possibly deleterious variants of DDR genes can lead to impaired function of DDR proteins (29,36). Therefore, it is of great clinical application value to find a biomarker for predicting the efficacy of platinum chemotherapy at the DNA level. It is meaningful to explore the correlation between DDR gene mutations and the efficacy of PC and IPC in advanced NSCLC patients.

In this study, we attempted to determine whether DDR gene alterations were associated with increased sensitivity to PC and IPC among advanced NSCLC patients. We present the following article in accordance with the REMARK reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-746/rc).

Methods

Study design and patients

As shown in *Figure 1*, from October 2016 to September 2021, patients diagnosed with unresectable locally advanced or metastatic NSCLC and underwent tissue-based targeted exon sequencing prior to starting treatment were included from the First Affiliated Hospital of Guangzhou University of Chinese Medicine. (I) All patients were retrospectively collected and divided into two groups based on the presence of DDR pathway mutations. (II) We compared patients with DDR mutations to those without mutations in terms of genomic landscape, TMB, and PD-L1 levels. (III) After excluding patients who did not receive PC or IPC and those whose treatment was ambiguous, we also explored whether DDR mutations are associated with objective response rates (ORRs), disease control rates (DCRs) at 6 months after PC or IPC, progression-free survival (PFS), and overall survival (OS) in the PC and IPC group. (IV) We also assessed the predictive power of different DDR pathways by dividing DDR genes into different pathways and by comparing the PFS and OS of patients with specific DDR pathway

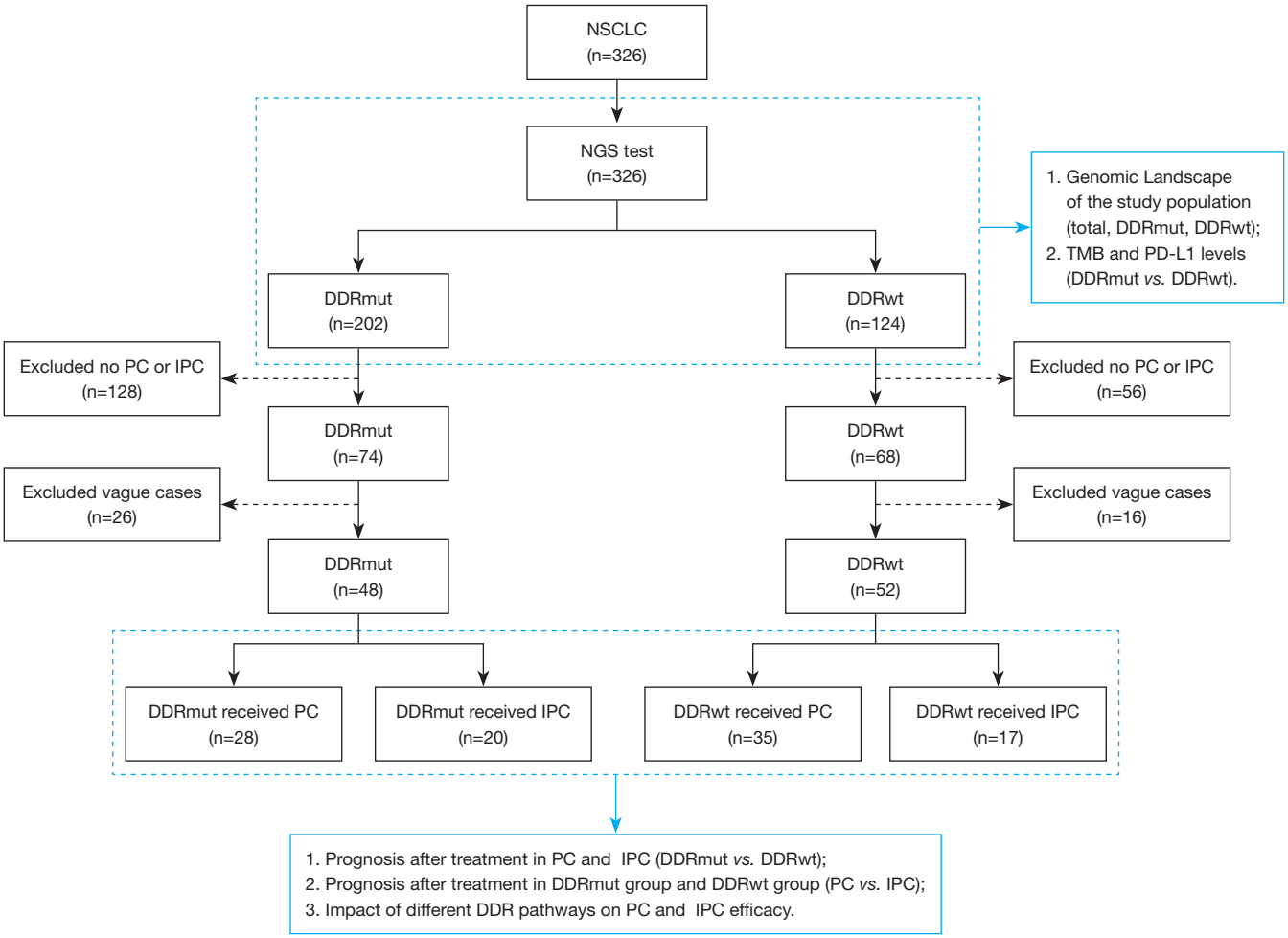

Figure 1 Flow diagram of the study. NSCLC, non-small cell lung cancer; NGS, next-generation sequencing; DDRmut, DNA damage repair mutations; DDRwt, DNA damage repair wild-type; PC, platinum-based chemotherapy; IPC, immunotherapy plus platinum-based chemotherapy.

mutations with those without any DDR gene mutations in the PC or IPC group.

In order to distinguish between DDR alterations' prognostic role and their function as a predictor of treatment efficacy, data from untreated advanced NSCLC patients in The Cancer Genome Atlas (TCGA) database were analyzed. The TCGA dataset was accessed via cBioPortal (http://www.cbioportal.org/).

The primary endpoints of the study were to explore whether DDR mutations are associated with ORRs, DCRs, PFS, and OS of PC and IPC. The secondary end point was to explore the association between DDR mutations and the choice to add immunotherapy to chemotherapy, and the impact of different DDR pathways on efficacy in PC and IPC. An exploratory objective of this study was to identify genomic and immunologic features between patients with DDR mutations and those without mutations.

Clinical data about age, gender, Eastern Cooperative Oncology Group (ECOG) performance status, histology, smoking history, tumor blood markers, comorbidities, sites of metastatic disease, systematic treatment program, and so on, were collected from the patient medical records. Telephone follow-up and outpatient records were used for survival data, and the cutoff date for follow-up of the current study was November 2021.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Collection and analysis of data were approved by the Ethics Committee of

the First Affiliated Hospital of Guangzhou University of Chinese Medicine (No. K-2022-118). The requirement for informed consent was waived because patients, at the time of treatment, had consented to their anonymized medical data being analyzed and published for research purposes.

Clinical outcomes

Scans were interpreted by a dedicated chest radiologist using RECIST version 1.1 to determine ORR, DCR, and PFS (37). The PFS was calculated from the start date of PC or IPC to the date of progression, death, or last follow-up. The OS was calculated from the start date of PC to the date of death or the last follow up. At the time of last contact, patients who were still alive were examined. Patients still alive at last follow-up were censored for OS. Patients alive and without progression were censored for PFS.

Tumor tissue-based next generation sequencing

Formalin-fixed paraffin-embedded (FFPE) samples for the detection of genetic alterations were taken from needle biopsies of unresectable locally advanced or metastatic patients. All samples were independently confirmed by pathologists as consistent with the morphological characteristics of NSCLC and genomic profiling was performed in 3D Medicines Laboratory (3D Medicines Inc., Shanghai, China). The patient-generated libraries were loaded into the NovaSeq 6000 platform (Illumina, San Diego, CA, USA) for 100 bp peer sequencing with an average sequencing depth of ×1,000. The Burrows-Wheeler Aligner was used to map raw data from tumor and normal tissue paired samples to the reference human genome hg19 (BWA; version 0.7.12) (38). Picard (version 1.130; Broad Institute, Cambridge, MA, USA) was used to remove Polymerase chain reaction (PCR) repeats, and collect sequence measurements using SAMtools (version 1.1.19; http://www.htslib.org/). The variants were called only in the target area. Somatic single nucleotide variations (SNVs) were detected using an internally developed R package that detects variation based on a binomial test. Perform local rearrangement to detect inserts and deletions (indels). The variables were then filtered based on their unique support for read depth, chain bias, and base quality (39). All variants were then screened using automatic false positive tests screening pipeline to ensure sensitivity and specificity for allele frequencies ≥5%. Single nucleotide polymorphisms (SNPs) and indels were indexed with ANNOVAR (https://annovar.openbioinformatics.org/en/latest/) to note: the following database dbSNP (version 138), 1000 genomes and ESP6500 (group frequency >0.015). Only missense, stopgain, frameshift, and non-frameshift indel mutations were retained. Copy number variations (CNVs) and gene rearrangements were detected as described (39).

Targeted next-generation sequencing (NGS) was performed using a panel covering the exons of 381 cancer-related genes on an Illumina NextSeq 550 instrument (Table S1). A total of 35 genes were identified as DDR pathway genes based on searches of the PubMed, National Center for Biotechnology Information (NCBI) Gene, and NCBI BioSystems databases (Table S2).

Determination of deleterious DDR mutation status, tumor mutation burden (TMB) and PD-L1

All loss-of-function mutations in DDR genes were considered deleterious, including nonsense mutations, frameshift, or splice site alterations. To determine functional impact of missense mutations, we employed two different approaches. First, we performed an in silico functional analysis using the PolyPhen-2 (40) prediction tool to determine the functional significance of each missense mutation. Second, we reviewed all the identified missense mutations in the Catalogue of Somatic Mutations in Cancer (COSMIC) (41) and ClinVar (42) databases.

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 mutations (DDRmut), and those without deleterious DDR mutations were defined as the DDR wild-type (DDRwt) subgroup.

TMB was defined as non-synonymous somatic SNVs and indels number of enzymes per megabyte that mutations in the detected coding region. All SNVs and indels in the coding region of targeted genes were considered, including missense, silent, stop gain, stop loss, in-frame, and frameshift mutations. Microsatellite instability (MSI) was assessed at 100 microsatellite loci and MSI scores for each analysis were calculated using the top 30 loci with the best coverage. An internally developed R package was used to assess the distribution of readings counting at different repeat lengths at each microsatellite site. A sample with an MSI score of at least 0.4 is considered to have high instability. Otherwise, they are considered to exhibit stability.

Table 1 Baseline patient characteristics

Characteristics	Total (n=326)	DDRmut (n=202)	DDRwt (n=124)	P value
Age, years				0.066
Mean (SD)	61.64 (10.80)	62.51 (10.69)	60.22 (11.23)	
Range	28–87	28–87	29–87	
Gender (%)				0.058
Female	112 (34.40)	61 (30.20)	51 (41.10)	
Male	214 (65.60)	141 (69.80)	73 (58.90)	
Treatment (%)				0.471
PC	63 (19.33)	28 (13.86)	35 (28.23)	
IPC	37 (11.35)	20 (9.90)	17 (13.71)	
Other treatments	226 (69.32)	154 (76.24)	72 (58.06)	

DDRmut, DNA damage repair mutations; DDRwt, DNA damage repair wild type; PC, platinum-based chemotherapy; IPC, immunotherapy plus platinum-based chemotherapy; SD, standard deviation.

PD-L1 immunohistochemistry (IHC) 22C3 pharmDx assay (Agilent Technologies Inc., Santa Clara, CA, USA) or PD-L1 IHC SP263 (Roche Diagnostics, Mannheim, Baden-Wurttemberg, Germany) to evaluate PD-L1 expression in FFPE tissue slices. The staining for 22C3 was performed on the Dako Link-48 automatic staining system at Teddy Clinical Research lab (Shanghai, China) and staining for SP263 was performed on the Roche BenchMark Ultra platform at the QIAGEN Suzhou Clinical Laboratory. PD-L1 expression was measured using the tumor proportion score (TPS), which is the proportion of surviving tumor cells with partial or full membrane PD-L1 staining at any intensity. PD-L1 was positive for TPS ≥1%.

Statistical analysis

The associations between continuous variables were calculated using the Wilcoxon rank-sum test and Kruskal-Wallis test, and the χ^2 test or Fisher's exact test was used to test for associations between categorical variables. The best response was assessed based on RECIST v1.1 criteria (https://recist.eortc.org/recist-1-1-2/). The Kaplan-Meier method was used to estimate OS, PFS; the log-rank test was used to compare differences; hazard ratios (HRs) were calculated using univariate Cox regression analysis. The Cox proportional hazards regression model was used to estimate the HRs of clinicopathological factors in univariate and multivariate analyses. Trend tests were conducted using logistic regression when the outcome was binary or linear regression with log-transformation for continuous outcomes.

All P values were two-sided with statistical significance defined as P≤0.05. All statistical analyses were performed using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA) and R software version 3.6.0 (The R Foundation for Statistical Computing, Vienna, Austria).

Results

Baseline characteristics of the total NSCLC population

As shown in *Figure 1*, a total of 326 NSCLC patients who had undergone NGS testing were obtained from the First Affiliated Hospital of Guangzhou University of Chinese Medicine between October 2016 to September 2021. Among these cases, the median age was 61.64 years (range, 28 to 87 years), and the ratio of male to female patients was 1.9:1 (214:112). In treatment after NGS testing, 63 (19.33%) advanced cases received PC (NGS test data of patients in the PC group are shown in available online: https://cdn.amegroups.cn/static/public/tlcr-22-746-1.pdf) and 37 (11.35%) advanced cases received IPC (NGS test data of patients in the IPC group are shown in available online: https://cdn.amegroups.cn/static/public/tlcr-22-746-2.pdf); 226 (69.32%) advanced cases received other treatments. The baseline characteristics of the patients are shown in (*Table 1*).

Genomic landscape of the NSCLC population

In the main (326 cases) NSCLC population, *TP53* had the highest mutation frequency (58.90%) followed by *EGFR* (35.28%) (Figure S1), which was similar to the results of previous study (43). Some 202 cases (61.96%) harboured DDRmut, with a median age of 62.51 years and the ratio of male to female patients was 2.3:1 (141:61, *Table 1*).

To obtain a comprehensive molecular understanding of the DDRmut cases, we investigated the genomic landscape in both DDRmut and DDRwt group patients. In the DDRmut group (202 patients), *TP53* had the highest mutation frequency (62.38%) followed by *EGFR* (36.63%), *LRP1B* (18.32%), *ATM* (16.34%), *KRAS* (16.34%), and so on (*Figure 2A*). In the DDRwt group (124 patients), the five most frequently mutated genes were identified, including *TP53* (53.23%), *EGFR* (33.06%), *KRAS* (14.52%), *CDKN2A* (12.10%), and *LRP1B* (12.10%), for which the mutation frequencies were similar but lower than those in the DDRmut group (*Figure 2B*). In addition, the most frequently mutated DDR genes in the DDRmut group were *ATM* (16.34%), BRCA2 (12.39%), *PTEN* (7.43%), and *SMARCA4* (6.44%) (Figure S2).

For the association of DDRmut and immune biomarkers, TMB was measured in 171 patients (120 in the DDRmut group and 51 in the DDRwt group), and PD-L1 was assessed in 134 patients (97 in the DDRmut group and 37 in the DDRwt group). The median TMB of the DDRmut group was significantly higher than that of the DDRwt group (7.5419 *vs.* 5.58659 muts/Mb, P=0.0008) (*Figure 3A*). The rate of strong PD-L1 (TPS ≥50%) positivity of the DDRmut group was numerically higher than that of the DDRwt group (29.03% *vs.* 20.00%) (*Figure 3B*).

The association of DDR mutations and outcomes after PC

The baseline characteristics of the patients undergoing PC are shown in (*Table 2*). In 63 patients undergoing PC, the ORRs were 15.38% for the DDRmut group and 2.86% for the DDRwt group (P=0.15358), and the DCRs were 88.46% for the DDRmut group and 45.72% for the DDRwt patients (P=0.00097) at 6 months (*Figure 4A*). The median PFS (mPFS) of the total population was 5.07 months (Figure S3A), which was similar to the results of previous study (mPFS: 3.6 months) (16). The DDRmut patients displayed a significantly better mPFS than the DDRwt patients [7.6 *vs.* 3.9 months, HR =1.93, 95% confidence interval (CI): 1.09 to 3.41, P=0.0220, *Figure 4B*]. The mOS of the total population was 28.2 months (Figure S3B). The DDRmut patients also displayed a significantly better mOS than the DDRwt patients (29.9 *vs.* 20.7 months, HR =2.31, 95% CI: 1.09 to 4.9, P=0.0250, *Figure 4C*).

When patients carrying driver mutations in *EGFR*, *ALK*, *ROS1*, and *RET* were excluded, the mPFS of the DDRmut group was not significantly longer than that of the DDRwt patients (5 *vs.* 3.43 months, HR =1.72, 95% CI: 0.84 to 3.53, P=0.1300; *Figure 4D*), yet mOS remained significantly improved (53.4 *vs.* 11.5 months, HR =2.84, 95% CI: 1.09 to 7.4, P=0.0270; *Figure 4E*). Comparing DDRmut with DDRwt patients carrying driver mutations, there was a significant difference in mPFS (16.83 *vs.* 5.07 months, HR =2.73, 95% CI: 0.98 to 7.58, P=0.046; *Figure 4F*), but no significant differences in mOS (47.6 *vs.* 29.4 months, HR =1.52, 95% CI: 0.43 to 5.33, P=0.5100; *Figure 4G*).

In univariate analyses, deficiency in DDR genes was significantly correlated with PFS (*Table 3*) and OS (*Table 4*). In multivariate analyses, mutations in DDR genes remained a predictor of PFS and OS. Lymphatic metastases, and *RET* mutation were also significantly correlated with OS (*Table 4*). In addition, we analyzed the relationship between DDR gene mutations and the efficacy of different types of platinum agents. It was observed that the predictive effects of DDR mutations were similar for both PFS (*Table 3*) and OS (*Table 4*) between patients treated with carboplatin and cisplatin.

The association of DDR mutations and outcomes after IPC

In 37 patients undergoing IPC, the ORRs were 45.00% for the DDRmut group and 11.76% for the DDRwt group (P=0.03646), and the DCRs were 95.00% for the DDRmut group and 70.58% for the DDRwt group at 6 months (P=0.07523) (*Figure 5A*). The mPFS of the total population was 8.8 months (Figure S3C), which was similar to the results of previous study (mPFS: 8.8 months) (44). The DDRmut cases displayed a significantly better mPFS than the DDRwt patients (19.5 *vs.* 4.5 months, HR =3.28, 95% CI: 1.53 to 9.56, P=0.0022, *Figure 5B*).

When patients carrying driver mutations in *EGFR*, *ALK*, *ROS1*, and *RET* were excluded, the mPFS of the DDRmut group was significantly longer than that of the DDRwt patients (19.5 *vs.* 4.5 months, HR =3.34, 95% CI: 1.1 to 10.15, P=0.0250; *Figure 5C*). The mPFS had no significant differences for the DDRmut patients over their wild-type counterparts in patients carrying driver mutations

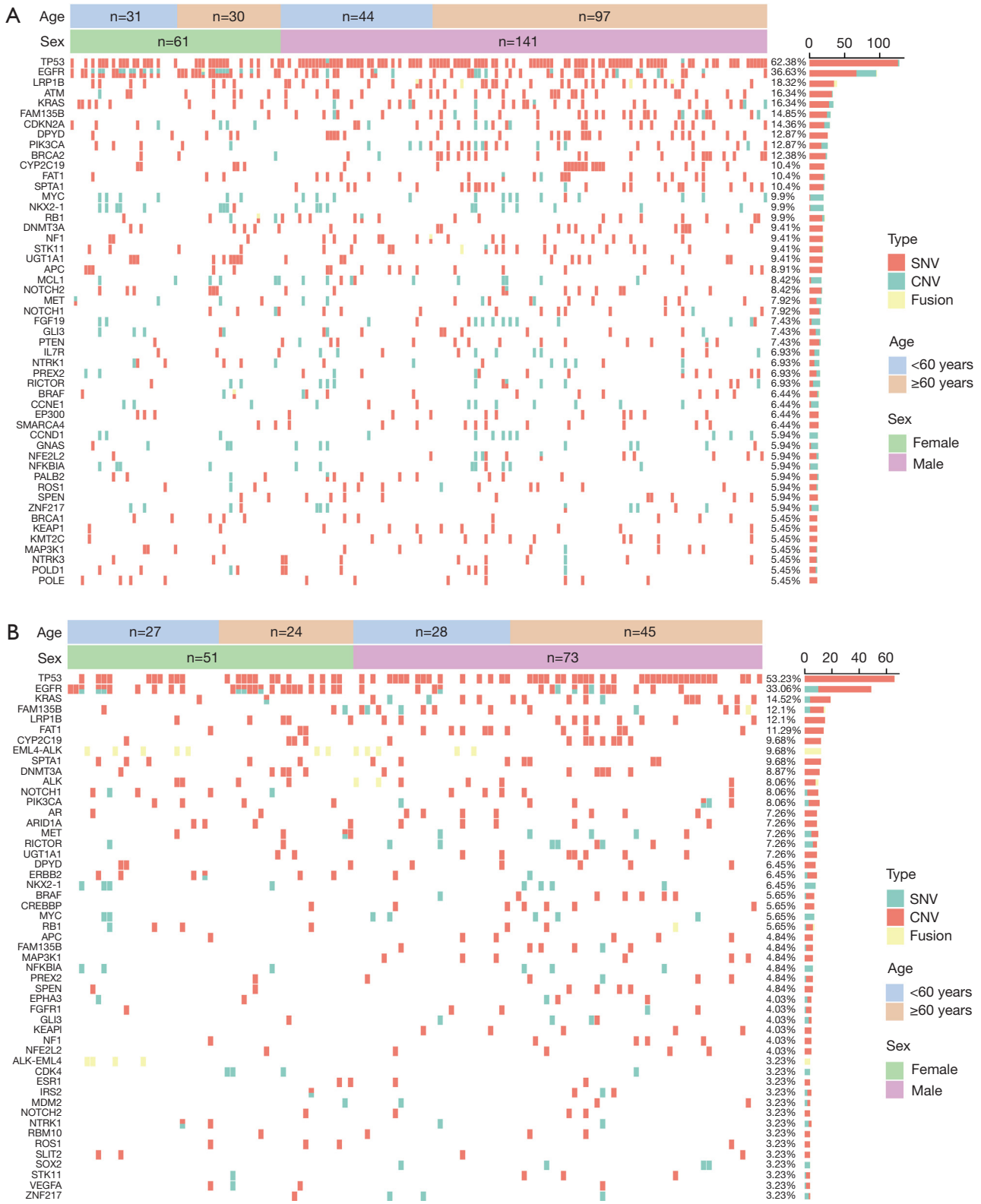

Figure 2 Genomic landscape of (A) DDRmut and (B) DDRwt group patients. SNV, single nucleotide variants; CNV, copy number variations; DDRmut, DNA damage repair mutations; DDRwt, DNA damage repair wild-type.

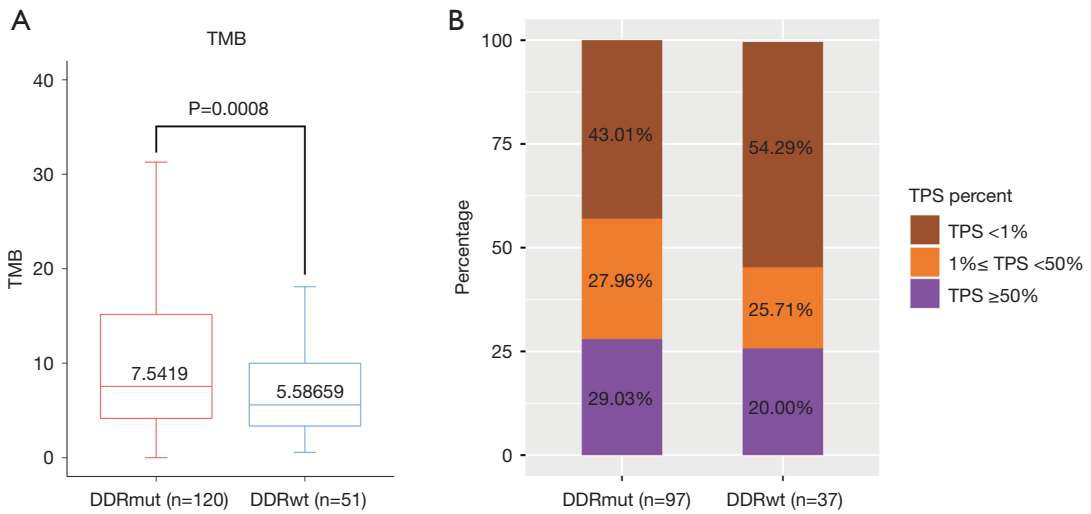

Figure 3 (A) TMB and (B) PD-L1 level between DDRmut and DDRwt group. TMB, tumor mutational burden; DDRmut, DNA damage repair mutations; DDRwt, DNA damage repair wild-type; TPS, tumor proportion score; PD-L1, programmed cell death-ligand 1.

(NA *vs.* 6.5 months, HR =3.68, 95% CI: 0.72 to 18.79, P=0.0950; *Figure 5D*). The baseline characteristics of the patients undergoing IPC are shown in (*Table 2*).

In univariate analyses, DDR gene mutations were significantly correlated with PFS as well as multivariate analyses. Lymphatic metastasis was significantly correlated with PFS only in univariate analyses (*Table 5*). The predictive effects of DDR mutations were similar for PFS with different types of platinum agents. Patients receiving IPC in later lines (3rd line and beyond) had a worse PFS than those receiving it in earlier lines (1st to 2nd line) (*Table 5*).

The association of DDR mutations and the choice to add immunotherapy to chemotherapy

In order to better identify patients who would benefit from the addition of immunotherapy, we compared the correlation between DDR mutations and outcomes on IPC and PC. For cases in the DDRmut group, the mPFS of IPC was 19.5 months, which was significantly better than that of PC (7.6 months, HR =2.09, 95% CI: 0.98 to 4.42, P=0.0500, *Figure 6A*). However, the mPFS of IPC and PC was not significantly different in the DDRwt group (4.5 *vs.* 3.9 months, HR =1.14, 95% CI: 0.58 to 2.24, P=0.7100) (*Figure 6B*).

Similarly, when patients carrying driver mutations in *EGFR*, *ALK*, *ROS1*, and *RET* were excluded, the mPFS of those receiving IPC was significantly longer than that of those receiving PC in the DDRmut group (19.5 *vs.* 5 months, HR =2.6, 95% CI: 1.01 to 6.71, P=0.0400; *Figure 6C*). However, there were no significant differences in mPFS between the cases undergoing either IPC or PC in DDRwt group (4.5 *vs.* 3.43 months, HR =1.48, 95% CI: 0.64 to 3.43, P=0.3500; *Figure 6D*). Among patients carrying driver mutations, the mPFS was not significantly different between IPC and PC in both the DDRmut (16.8 *vs.* NA months, HR =1.7, 95% CI: 0.42 to 6.83, P=0.4500, *Figure 6E*) and DDRwt (6.5 *vs.* 5.07 months, HR =0.95, 95% CI: 0.26 to 3.52, P=0.9400, *Figure 6F*) groups. These findings suggest IPC mainly benefits patients who are driver oncogene negative, and DDRmut.

Impact of different DDR pathways on efficacy in PC and IPC

To further explore the correlation between DDRmut and efficacy, DDRmut patients were divided into three categories according to the pathway of genes, including HRR single pathway mutations (HRR), HRR combined with other pathway mutations (HRR comutations), and non-HRR pathway mutations (others), since HRR pathway gene mutations accounted for the highest proportion in DDR pathways.

The results showed that the alterations of some DDR pathways showed better efficacy. Efficacy of PC were more pronounced with patients with mutations in the HRR single pathway (HR =0.27; 95% CI: 0.06 to 1.18, P=0.064, *Figure 7A*) and non-HRR pathway (HR =0.37;

Table 2 Baseline patient characteristics of the platinum-based chemotherapy and immunotherapy plus platinum-based chemotherapy groups

Treatment method	Platinum-based chemotherapy				Immunotherapy plus platinum-based chemotherapy			
	Total (n=63)	DDRmut (n=28)	DDRwt (n=35)	P value	Total (n=37)	DDRmut (n=20)	DDRwt (N=17)	P value
Age, years				0.149				0.511
Mean (SD)	59.38 (8.40)	61.11 (8.69)	58.00 (8.15)		59.38 (11.88)	60.6 (10.02)	57.94 (14.25)	
Range	43–79	43–79	43–75		30–80	32–73	30–80	
Gender (%)				0.348				0.069
Female	16 (24.65)	5 (17.90)	11 (31.40)		9 (25.60)	2 (10.00)	7 (41.20)	
Male	47 (75.35)	23 (82.10)	24 (68.60)		28 (74.40)	18 (90.00)	10 (58.80)	
ECOG (%)				0.382				0.373
1	52 (83.20)	25 (89.30)	27 (77.10)		30 (80.75)	17 (85.00)	13 (76.50)	
2	10 (15.35)	3 (10.70)	7 (20.00)		6 (16.75)	2 (10.00)	4 (23.50)	
4	1 (1.45)	0 (0.00)	1 (2.90)		1 (2.50)	1 (5.00)	0 (0.00)	
Histology (%)				0.603				0.262
LCLC	2 (3.25)	1 (3.60)	1 (2.90)		0 (0.00)	0 (0.00)	0 (0.00)	
LUAD	51 (81.40)	24 (85.70)	27 (77.10)		26 (71.20)	12 (60.00)	14 (82.40)	
LUSC	10 (15.35)	3 (10.70)	7 (20.00)		11 (28.80)	8 (40.00)	3 (17.60)	
Smoke (%)				0.799				0.748
N-Miss	2	0	2					
No	37 (60.35)	16 (57.10)	21 (63.60)		26 (69.85)	15 (75.00)	11 (64.70)	
Yes	24 (39.65)	12 (42.90)	12 (36.40)		11 (30.15)	5 (25.00)	6 (35.30)	
EGFR (%)				1.000				0.482
Wild type	48 (76.05)	21 (75.00)	27 (77.10)		33 (88.70)	19 (95.00)	14 (82.40)	
Mutation	15 (23.95)	7 (25.00)	8 (22.90)		4 (11.30)	1 (5.00)	3 (17.60)	
ALK (%)				0.184				0.818
Wild type	59 (94.30)	28 (100.00)	31 (88.60)		31 (84.10)	16 (80.00)	15 (88.20)	
Mutation	4 (5.70)	0 (0.00)	4 (11.40)		6 (15.90)	4 (20.00)	2 (11.80)	
ROS1 (%)				1.000				0.720
Wild type	57 (90.35)	25 (89.30)	32 (91.40)		33 (89.55)	17 (85.00)	16 (94.10)	
Mutation	6 (9.65)	3 (10.70)	3 (8.60)		4 (10.45)	3 (15.00)	1 (5.90)	
RET (%)				1.000				1.000
Wild type	62 (98.55)	28 (100.00)	34 (97.10)		35 (94.55)	19 (95.00)	16 (94.10)	
Mutation	1 (1.45)	0 (0.00)	1 (2.90)		2 (5.45)	1 (5.00)	1 (5.90)	
CEA (%)				0.033				0.409
N-Miss	15	5	10		3	1	2	
<5	29 (61.15)	18 (78.30)	11 (44.00)		24 (69.45)	15 (78.90)	9 (60.00)	
≥5	19 (38.85)	5 (21.70)	14 (56.00)		10 (30.55)	4 (21.10)	6 (40.00)	

Table 2 (*continued*)

Table 2 (*continued*)

Treatment method	Platinum-based chemotherapy				Immunotherapy plus platinum-based chemotherapy			
	Total (n=63)	DDRmut (n=28)	DDRwt (n=35)	P value	Total (n=37)	DDRmut (n=20)	DDRwt (N=17)	P value
CFRA21_1 (%)				1				0.211
N-Miss	36	18	18		12	5	7	
<3.3	14 (51.45)	5 (50.00)	9 (52.90)		10 (36.65)	8 (53.30)	2 (20.00)	
≥3.3	13 (48.55)	5 (50.00)	8 (47.10)		15 (63.35)	7 (46.70)	8 (80.00)	
SCC (%)				1				1.000
N-Miss	28	15	13		14	8	6	
<1.5	26 (74.80)	10 (76.90)	16 (72.70)		18 (78.40)	9 (75.00)	9 (81.80)	
≥1.5	9 (25.20)	3 (23.10)	6 (27.30)		5 (21.60)	3 (25.00)	2 (18.20)	
NSE (%)				0.410				0.368
N-Miss	31	8	23		13	7	6	
<16.3	17 (55.85)	8 (66.70)	9 (45.00)		14 (59.45)	6 (46.20)	8 (72.70)	
≥16.3	15 (44.15)	4 (33.30)	11 (55.00)		10 (40.55)	7 (53.80)	3 (27.30)	
Hypertension (%)				0.299				1.000
N-Miss	3	1	2					
No	47 (77.60)	19 (70.40)	28 (84.80)		29 (78.25)	16 (80.00)	13 (76.50)	
Yes	13 (22.40)	8 (29.60)	5 (15.20)		8 (21.75)	4 (20.00)	4 (23.50)	
Digestive ulcer (%)				N/A				0.934
N-Miss	3	1	2					
No	60 (100.00)	27 (100.00)	33 (100.00)		36 (97.05)	20 (100.00)	16 (94.10)	
Yes	0 (0.00)	0 (0.00)	0 (0.00)		1 (2.95)	0 (0.00)	1 (5.90)	
Cardiovascular disease (%)				0.489				1.000
N-Miss	3	1	2					
No	54 (89.55)	23 (85.20)	31 (93.90)		32 (86.60)	17 (85.00)	15 (88.20)	
Yes	6 (10.45)	4 (14.80)	2 (6.10)		5 (13.40)	3 (15.00)	2 (11.80)	
Cerebrovascular disease (%)				0.919				0.363
N-Miss	3	1	2					
No	59 (98.15)	26 (96.30)	33 (100.00)		36 (97.30)	20 (100.00)	16 (94.10)	
Yes	1 (1.85)	1 (3.70)	0 (0.00)		1 (2.70)	0 (0.00)	1 (5.90)	
Other comorbidity (%)				1.000				0.716
No	41 (65.00)	18 (64.30)	23 (65.70)		24 (64.40)	14 (70.00)	10 (58.80)	
Yes	22 (35.00)	10 (35.70)	12 (34.30)		13 (35.60)	6 (30.00)	7 (41.20)	

Table 2 (*continued*)

Table 2 (*continued*)

Treatment method	Platinum-based chemotherapy				Immunotherapy plus platinum-based chemotherapy			
	Total (n=63)	DDRmut (n=28)	DDRwt (n=35)	P value	Total (n=37)	DDRmut (n=20)	DDRwt (N=17)	P value
Visceral metastasis (%)				0.179				N/A
N-Miss	8	1	7					
No	18 (32.55)	6 (22.20)	12 (42.90)		0 (0.00)	0 (0.00)	0 (0.00)	
Yes	37 (67.45)	21 (77.80)	16 (57.10)		37 (100.00)	20 (100.00)	17 (100.00)	
Brain metastasis (%)				0.631				1.000
N-Miss	8	1	7					
No	49 (89.05)	23 (85.20)	26 (92.90)		31 (83.70)	17 (85.00)	14 (82.40)	
Yes	6 (10.95)	4 (14.80)	2 (7.10)		6 (16.30)	3 (15.00)	3 (17.60)	
Osseous metastasis (%)				0.661				0.005
N-Miss	8	1	7					
No	47 (85.40)	22 (81.50)	25 (89.30)		19 (49.25)	15 (75.00)	4 (23.50)	
Yes	8 (14.60)	5 (18.50)	3 (10.70)		18 (50.75)	5 (25.00)	13 (76.50)	
Hepatic metastasis (%)				0.985				1.000
N-Miss	8	1	7					
No	54 (98.18)	26 (96.30)	28 (100.00)		31 (83.70)	17 (85.00)	14 (82.40)	
Yes	1 (1.82)	1 (3.70)	0 (0.00)		6 (16.30)	3 (15.00)	3 (17.60)	
Lymphatic metastasis (%)				1.000				0.934
N-Miss	8	1	7					
No	33 (60.00)	11 (91.70)	30 (81.10)		1 (2.70)	0 (0.00)	1 (5.88)	
Yes	22 (40.00)	1 (8.30)	7 (18.90)		36 (97.30)	20 (100.00)	16 (94.12)	
Treatment line (%)				0.644				0.085
1	59 (93.65)	12 (92.31)	41 (91.10)		17 (45.95)	10 (50.00)	7 (41.20)	
2	1 (1.59)	0 (0.00)	1 (2.20)		11 (29.73)	8 (40.00)	3 (17.60)	
3	2 (3.17)	1 (7.69)	2 (4.40)		7 (18.92)	1 (5.00)	6 (35.30)	
4	1 (1.59)	0 (0.00)	1 (2.20)		1 (2.70)	1 (5.00)	0 (0.00)	
5	0 (0.00)	0 (0.00)	0 (0.00)		1 (2.70)	0 (0.00)	1 (5.90)	
Platinum type (%)				0.038				0.246
Carboplatin	29 (46.03)	3 (18.75)	21 (46.67)		16 (43.24)	10 (50.00)	6 (35.30)	
Cisplatin	33 (52.38)	12 (75.00)	24 (53.33)		15 (40.54)	9 (45.00)	6 (35.30)	
Lobaplatin	1 (1.59)	1 (6.25)	0 (0.00)		6 (16.22)	1 (5.00)	5 (29.40)	

DDRmut, DNA damage repair mutations; DDRwt, DNA damage repair wild type; ECOG, Eastern Cooperative Oncology Group; LCLC, large cell lung cancer; LUAD, lung adenocarcinoma; LUSC, squamous cell lung carcinoma; N-Miss, the number of patients who lack this information; ALK, anaplastic lymphoma kinase; ROS1, ROS proto-oncogene 1; RET, RET proto-oncogene; CEA, carcinoembryonic antigen; CFRA21_1, cytokeratin 19 fragment antigen 21-1; SCC, squamous cell carcinoma antigen; NSE, neuronal-specific enolase; SD, standard deviation.

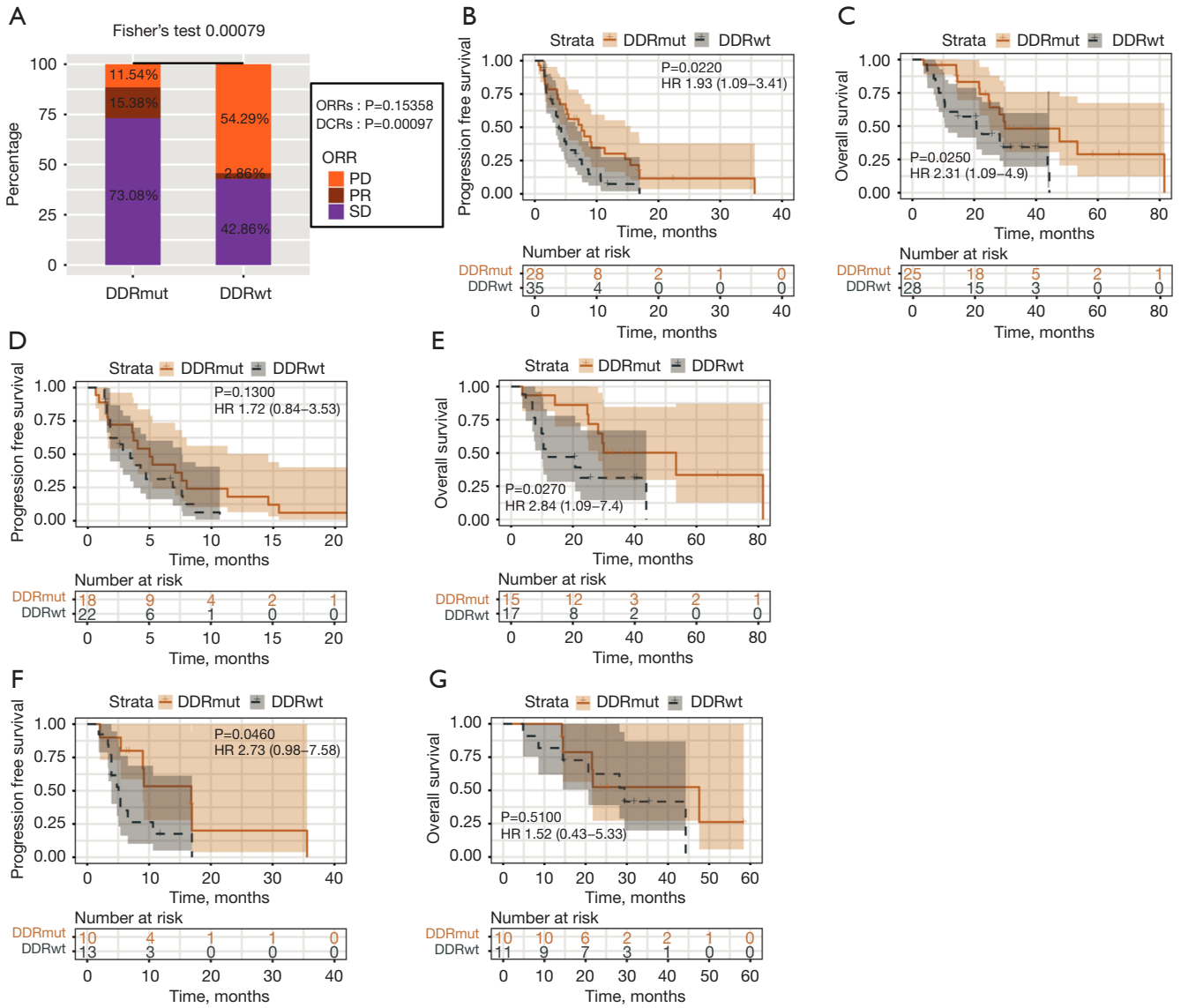

Figure 4 Efficacy and Kaplan-Meier survival curves of PC group between DDRmut and DDRwt patients. (A) ORRs and DCRs; (B) PFS in all patients receiving PC; (C) OS in all patients receiving PC; (D) PFS in patients receiving PC without carrying the driver genes; (E) OS in patients receiving PC carrying the driver genes; (F) PFS in patients receiving PC carrying driver mutations; (G) OS in in patients receiving PC carrying driver mutations. DDRmut, DNA damage repair mutations; DDRwt, DNA damage repair wild-type; ORRs, objective response rates; DCR, disease control rates; PD, progressive disease; PR, partial response; SD, stable disease; PC, platinum-based chemotherapy; PFS, progression-free survival; OS, overall survival.

95% CI: 0.14 to 1.01, P=0.043, *Figure 7A*) for OS, and non-HRR pathway (HR =0.44; 95% CI: 0.22 to 0.91, P=0.024, *Figure 7B*) in PFS. For patients who received IPC, a PFS benefit was most pronounced for patients with mutations in HRR combined with other pathways (HR =0.16; 95% CI: 0.03 to 0.74, P=0.0085) (*Figure 7C*).

The prognostic role of DDR alterations

We evaluated whether DDR status was a prognostic factor using the survival data and sequencing data of previously untreated NSCLC patients in the TCGA database. There was no significant difference in mOS between the DDRmut and DDRwt groups in either untreated stage I patients

Table 3 Univariable and multivariable analyses for progression-free survival of platinum-based chemotherapy group

Variables	Univariable analysis		Multivariable analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Age, years				
<60	1			
≥60	0.84 (0.49 to 1.46)	0.545		
Gender				
Female	1			
Male	1.50 (0.80 to 2.82)	0.211		
ECOG				
0–1	1			
≥2	0.93 (0.44 to 1.99)	0.860		
Histology				
Non-lung adenocarcinoma	1			
Lung adenocarcinoma	1.30 (0.62 to 2.71)	0.483		
Smoke				
No	1			
Yes	1.40 (0.79 to 2.48)	0.247		
DDR				
Mutation	1		1.00	
Wild type	1.93 (1.09 to 3.41)	0.024	3.67 (1.53 to 8.78)	0.004
EGFR				
Mutation	1			
Wild type	1.88 (0.94 to 3.75)	0.075		
ALK				
Mutation	1			
Wild type	0.84 (0.30 to 2.36)	0.747		
ROS1				
Mutation	1			
Wild type	1.98 (0.77 to 5.11)	0.156		
RET				
Mutation	1			
Wild type	0.33 (0.04 to 2.47)	0.279		
CEA				
<5 ng/mL	1			
≥5 ng/mL	1.71 (0.86 to 3.40)	0.128		

Table 3 (*continued*)

Table 3 (*continued*)

Variables	Univariable analysis		Multivariable analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
CFRA21_1				
<3.3 ng/mL	1			
≥3.3 ng/mL	0.81 (0.34 to 1.91)	0.634		
SCC				
<1.5 ng/mL	1			
≥1.5 ng/mL	2.59 (1.11 to 6.05)	0.028	2.41 (0.98 to 5.93)	0.055
NSE				
<16.3 ng/mL	1			
≥16.3 ng/mL	1.42 (0.65 to 3.09)	0.376		
Hypertension				
No	1			
Yes	1.39 (0.70 to 2.75)	0.349		
Cardiovascular disease				
No	1			
Yes	1.73 (0.68 to 4.41)	0.254		
Cerebrovascular disease				
No	1			
Yes	1.31 (0.18 to 9.63)	0.790		
Other comorbidity				
No	1			
Yes	1.29 (0.70 to 2.37)	0.413		
Visceral metastasis (total)				
No	1			
Yes	1.11 (0.60 to 2.05)	0.728		
Brain metastasis				
No	1			
Yes	1.01 (0.40 to 2.59)	0.979		
Osseous metastasis				
No	1			
Yes	0.56 (0.23 to 1.33)	0.190		
Hepatic metastasis				
No	1			
Yes	0.54 (0.07 to 4.00)	0.55		

Table 3 (*continued*)

Table 3 (*continued*)

Variables	Univariable analysis		Multivariable analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Lymphatic metastasis				
No	1			
Yes	1.87 (1.04 to 3.37)	0.036	2.30 (1.01 to 5.24)	0.048
PC line				
≤2	1			
≥3	0.36 (0.09 to 1.52)	0.165		
Platinum type				
Cisplatin	1			
Carboplatin	0.98 (0.56 to 1.71)	0.950		
Lobaplatin	0.54 (0.07 to 3.98)	0.542		

ECOG, Eastern Cooperative Oncology Group; DDR, DNA damage repair; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; ROS1, ROS proto-oncogene 1; RET, RET proto-oncogene; CEA, carcinoembryonic antigen; CFRA21_1, cytokeratin 19 fragment antigen 21-1; SCC, squamous cell carcinoma antigen; NSE, neuronal-specific enolase; PC, platinum-based chemotherapy; HR, hazard ratio; CI, confidence interval.

Table 4 Univariable and multivariable analyses for overall survival of platinum-based chemotherapy

Variables	Univariable analysis		Multivariable analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Age, years				
<60	1			
≥60	1.40 (0.68 to 2.86)	0.357		
Gender				
Female	1			
Male	0.96 (0.44 to 2.10)	0.917		
ECOG				
0–1	1			
≥2	0.70 (0.28 to 1.73)	0.437		
Histology				
Non-lung adenocarcinoma	1			
Lung adenocarcinoma	0.36 (0.11 to 1.18)	0.091		
Smoke				
No	1			
Yes	0.71 (0.33 to 1.51)	0.374		

Table 4 (*continued*)

Table 4 (*continued*)

Variables	Univariable analysis		Multivariable analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
DDR				
Mutation	1			
Wild type	2.31 (1.09 to 4.90)	0.030	5.32 (2.21 to 12.83)	0.0002
EGFR				
Mutation	1			
Wild type	1.14 (0.51 to 2.55)	0.755		
ALK				
Mutation	1			
Wild type	0.51 (0.18 to 1.49)	0.220		
ROS1				
Mutation	1			
Wild type	1.62 (0.38 to 6.87)	0.511		
RET				
Mutation	1			
Wild type	0.06 (0.01 to 0.57)	0.014	0.05 (0.00 to 0.49)	0.011
CEA				
<5 ng/mL	1			
≥5 ng/mL	1.38 (0.64 to 2.96)	0.412		
CFRA21_1				
<3.3 ng/mL	1			
≥3.3 ng/mL	0.71 (0.23 to 2.19)	0.550		
SCC				
<1.5 ng/mL	1			
≥1.5 ng/mL	2.23 (0.86 to 5.76)	0.099	2.41 (0.98 to 5.93)	0.055
NSE				
<16.3 ng/mL	1			
≥16.3 ng/mL	0.87 (0.34 to 2.26)	0.781		
Hypertension				
No	1			
Yes	1.06 (0.46 to 2.47)	0.889		
Cardiovascular disease				
No	1			
Yes	2.05 (0.70 to 5.99)	0.189		

Table 4 (*continued*)

Table 4 (*continued*)

Variables	Univariable analysis		Multivariable analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Cerebrovascular disease				
No	1			
Yes	1.17 (0.16 to 8.67)	0.880		
Other comorbidity				
No	1			
Yes	1.07 (0.49 to 2.32)	0.863		
Visceral metastasis (brain, osseous, hepatic, lymphatic)				
No	1			
Yes	1.16 (0.54 to 2.48)	0.709		
Brain metastasis				
No	1			
Yes	1.33 (0.46 to 3.86)	0.604		
Osseous metastasis				
No	1.00			
Yes	0.39 (0.11 to 1.33)	0.131		
Hepatic metastasis				
No	1			
Yes	0.00 (0.00 to Inf)	0.997		
Lymphatic metastasis				
No	1			
Yes	2.53 (1.22 to 5.26)	0.013	4.64 (2.02 to 10.69)	0.0003
PC line				
≤2	1			
≥3	0.62 (0.08 to 4.56)	0.637		
Platinum type				
Cisplatin	1			
Carboplatin	0.53 (0.25 to 1.13)	0.101		
Lobaplatin	0.85 (0.11 to 6.41)	0.873		

ECOG, Eastern Cooperative Oncology Group; DDR, DNA damage repair; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; ROS1, ROS proto-oncogene 1; RET, RET proto-oncogene; CEA, carcinoembryonic antigen; CFRA21_1, cytokeratin 19 fragment antigen 21-1; SCC, squamous cell carcinoma antigen; NSE, neuronal-specific enolase; PC, platinum-based chemotherapy; HR, hazard ratio; CI, confidence interval.

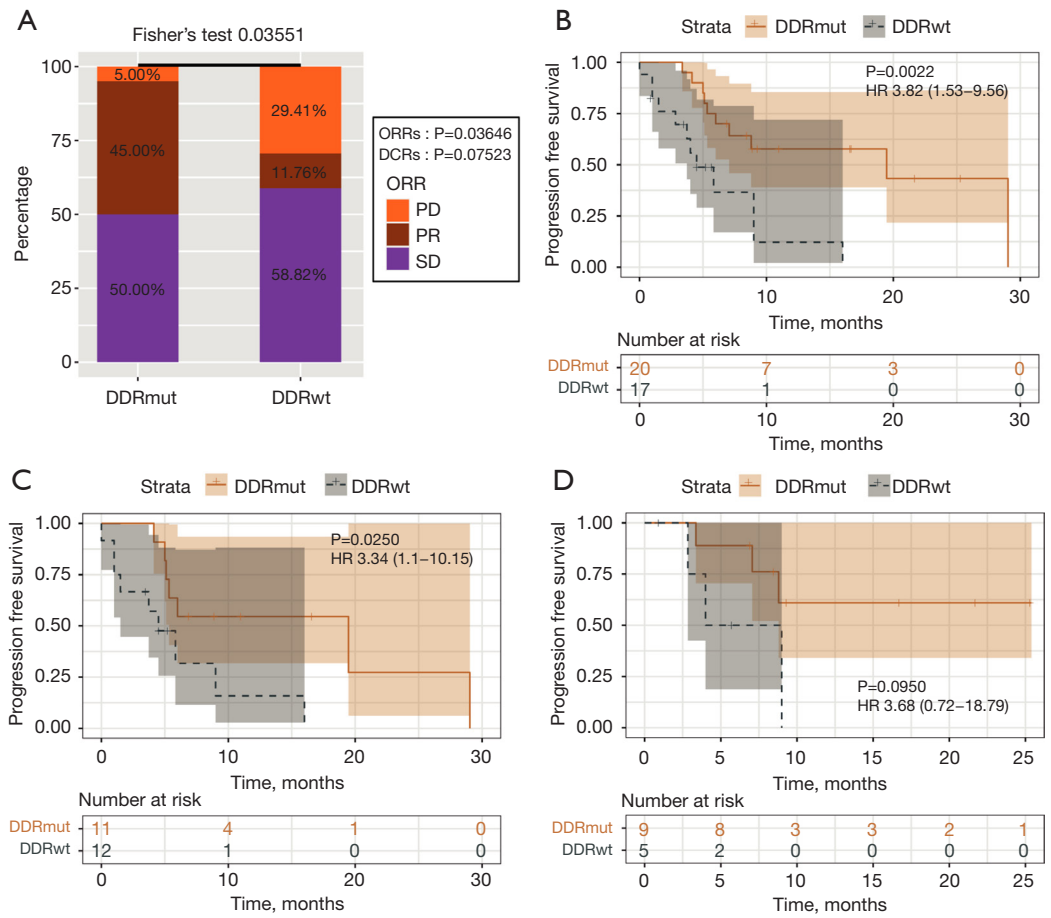

Figure 5 Efficacy and Kaplan-Meier survival curves of IPC group between DDRmut and DDRwt patients. (A) ORRs and DCRs; (B) PFS in all patients receiving IPC; (C) PFS in patients receiving IPC without carrying the driver genes; (D) PFS in patients receiving IPC carrying driver mutations. DDRmut, DNA damage repair mutations; DDRwt, DNA damage repair wild-type; ORRs, objective response rates; DCR, disease control rates; PD, progressive disease; PR, partial response; SD, stable disease; IPC, immunotherapy plus platinum-based chemotherapy; PFS, progression-free survival.

(DDRmut *vs.* DDRwt =86.1 *vs.* 75.7 months, HR =0.97; 95% CI: 0.67 to 1.41, P=0.8800; Figure S4A) or untreated stage II-IV patients (DDRmut *vs.* DDRwt =27.2 *vs.* 32.0 months, HR =0.81; 95% CI: 0.57 to 1.16, P=0.2500; Figure S4B). These results suggested that DDR alteration status was not a prognostic factor for untreated NSCLC.

Discussion

We examined the association between DDR mutations and clinical outcomes in two cohorts of NSCLC patients treated with PC and IPC respectively. We observed that 61.96% of tumors harbored alterations in DDR genes and that the presence of DDR gene variations was associated with improved ORRs, DCRs, PFS, and OS in NSCLC patients received PC or IPC. In the comparison of the efficacy of PC and IPC, patients with DDR mutations had a better efficacy of IPC, especially those without driver gene mutations. We also demonstrated that alterations of different DDR pathways had different influences on PFS and OS on PC and IPC.

DDR gene alterations are common in NSCLC but are poorly characterized. Polymorphisms in various DDR genes, such as *BRCA2* and *MLH3*, have been shown to be associated with leptomeningeal metastasis of NSCLC and associated with poor prognosis (45). To our knowledge,

Table 5 Univariable and multivariable analyses for progression-free survival of immunotherapy plus platinum-based chemotherapy

Variables	Univariable analysis		Multivariable analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Age, years				
<60	1			
≥60	1.03 (0.43 to 2.47)	0.940		
Gender				
Female	1			
Male	1.27 (0.37 to 4.35)	0.703		
ECOG				
0–1	1			
≥2	0.36 (0.13 to 0.98)	0.046	0.44 (0.16 to 1.22)	0.116
Histology				
Non-lung adenocarcinoma	1			
Lung adenocarcinoma	0.92 (0.35 to 2.41)	0.864		
Smoking				
No	1			
Yes	2.09 (0.85 to 5.16)	0.109		
DDR				
Mutation	1			
Wild type	3.82 (1.53 to 9.56)	0.004	3 (1.14 to 7.88)	0.026
EGFR				
Mutation	1			
Wild type	1.27 (0.17 to 9.67)	0.82		
ALK				
Mutation	1			
Wild type	0.84 (0.28 to 2.53)	0.753		
ROS1				
Mutation	1			
Wild type	4.41 (0.58 to 33.66)	0.153		
RET				
Mutation	1			
Wild type	0.00 (0.00 to Inf)	0.998		
CEA				
<5 ng/mL	1			
≥5 ng/mL	0.35 (0.08 to 1.54)	0.167		

Table 5 (*continued*)

Table 5 (*continued*)

Variables	Univariable analysis		Multivariable analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
CFRA21_1				
<3.3 ng/mL	1			
≥3.3 ng/mL	0.98 (0.33 to 2.96)	0.974		
SCC				
<1.5 ng/mL	1			
≥1.5 ng/mL	1.33 (0.27 to 6.66)	0.729		
NSE				
<16.3 ng/mL	1			
≥16.3 ng/mL	0.30 (0.06 to 1.47)	0.139		
Hypertension				
No	1			
Yes	1.07 (0.35 to 3.23)	0.904		
Digestive ulcer				
No	1			
Yes	4.48 (0.55 to 36.46)	0.161		
Cardiovascular disease				
No	1			
Yes	0.78 (0.18 to 3.38)	0.738		
Cerebrovascular disease				
No	1			
Yes	0.00 (0.00 to Inf)	0.999		
Other comorbidity				
No	1			
Yes	1.20 (0.48 to 3.00)	0.693		
Visceral metastasis (total)				
No	1			
Yes	NA	NA	NA	NA
Brain metastasis				
No	1			
Yes	0.79 (0.23 to 2.76)	0.712		
Osseous metastasis				
No	1			
Yes	1.99 (0.83 to 4.75)	0.122		

Table 5 (*continued*)

Table 5 (*continued*)

Variables	Univariable analysis		Multivariable analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Hepatic metastasis				
No	1			
Yes	0.79 (0.23 to 2.71)	0.713		
Lymphatic metastasis				
No	1			
Yes	0.05 (0.00 to 0.52)	0.013	0.12 (0.01 to 1.51)	0.102
IPC line				
≤2	1			
≥3	4.75 (1.73 to 13.06)	0.003	4.22 (1.38 to 12.87)	0.012
Platinum type				
Cisplatin	1			
Carboplatin	1.28 (0.49 to 3.35)	0.611		
Lobaplatin	2.18 (0.63 to 7.52)	0.217		

ECOG, Eastern Cooperative Oncology Group; DDR, DNA damage repair; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; ROS1, ROS proto-oncogene 1; RET, RET proto-oncogene; CEA, carcinoembryonic antigen; CFRA21_1, cytokeratin 19 fragment antigen 21-1; SCC, squamous cell carcinoma antigen; NSE, neuronal-specific enolase; IPC, immunotherapy plus platinum-based chemotherapy; HR, hazard ratio; CI, confidence interval.

this is the first study to demonstrate an independent association between DDR gene mutations and clinical benefit to PC in patients with advanced NSCLC, the results show that DDRmut patients displayed significantly better clinical outcomes than the DDRwt patients. Similarly, Li *et al.* reported that advanced NSCLC patients with downregulation of *APE1*, or *TUBB3* protein benefited from platinum plus paclitaxel chemotherapy (33). In a study on unresectable locally advanced or metastatic NSCLC patients treated with cisplatin-based chemotherapy, patients with deficient expression of *BRCA1* protein had a significantly higher survival rate than those with intact expression (34). A similar phenomenon was observed in our study cohort, where alteration of the *BRCA1* gene was significantly associated with better clinical outcomes (data not shown). However, a prospective study showed that *ERCC1* protein expression did not predict OS or PFS for NSCLC (46). The results suggested that IHC detection of *ERCC1* protein expression is not a good predictor of PC response, possibly because the reliability of IHC results was related to the quality of antibodies and subjective criteria for interpretation. On the contrary, the analysis of DDR genes using NGS can not only achieve high-throughput sequencing, but also ensure that the detection results are not affected by subjective interpretation factors of experimenters and can effectively distinguish deleterious and nondeleterious DDR gene mutations. In fact, DDR gene mutations have been shown to be associated with improved therapeutic sensitivity to PC, PARP inhibitors, ICIs, and other agents across multiple solid tumor types (29,47-49).

As early as 2018, Teo *et al.* reported the association between DDR gene and PD-L1 inhibitors monotherapy in advanced urethral carcinoma (50). A similar report had been made of NSCLC: DDR mutation patients had a significantly better ORRs, PFS, and OS than DDRwt with PD-L1 inhibitors monotherapy (51). Although the results of these studies indicate that patients with DDRmut can benefit from immunotherapy, patients in these studies were generally treated with immune monotherapy. As we know, immune monotherapy is recommended for patients whose PD-L1 expression exceeds 50% in NSCLC, so its clinical application is limited (7,8,52). In addition, PD-L1 combined with CTLA-4 inhibitor is still not a clinical preferred

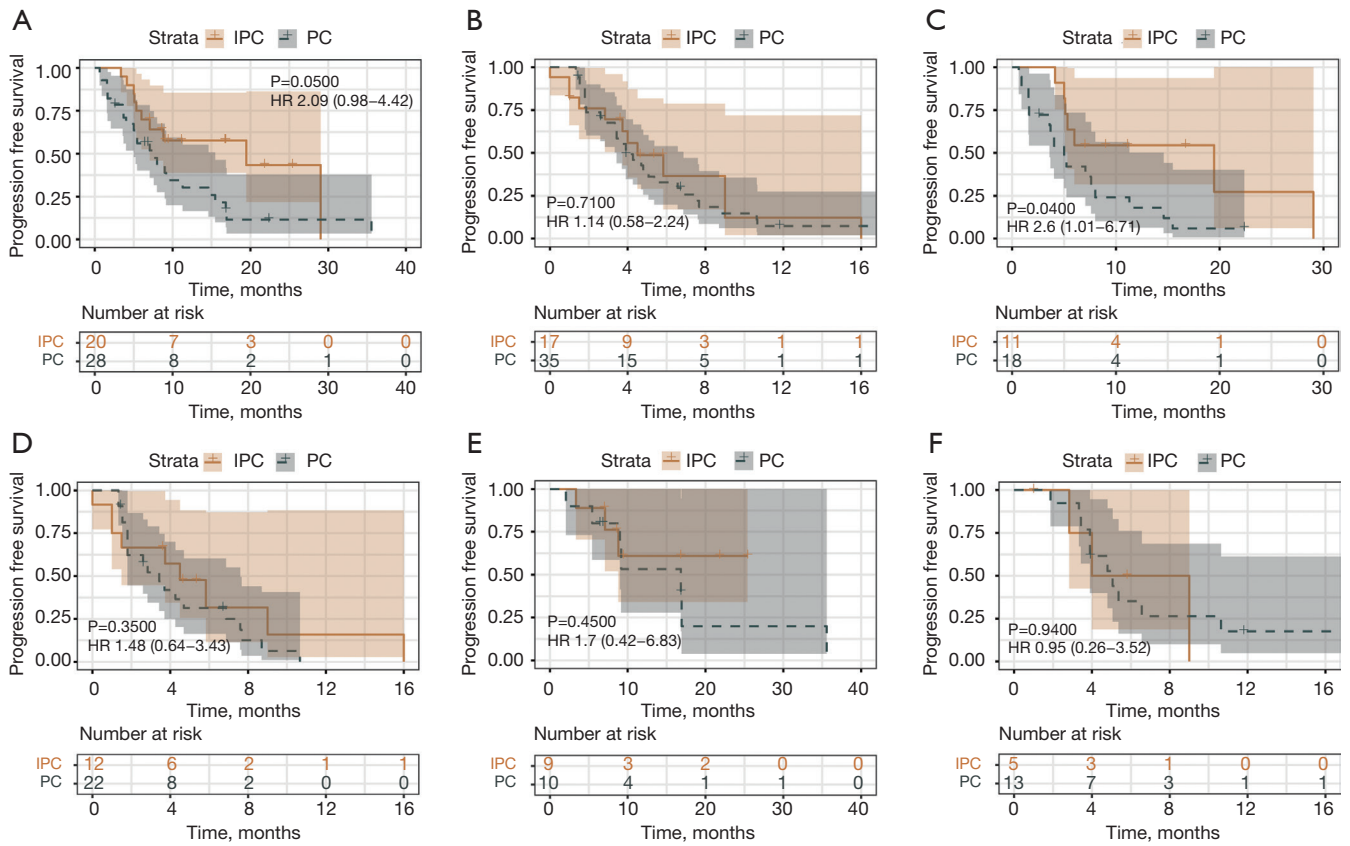

Figure 6 Kaplan-Meier survival curves of PFS between PC and IPC group. (A) PFS in all DDRmut patients; (B) PFS in all DDRwt patients; (C) PFS in DDRmut patients without carrying the driver genes; (D) PFS in all DDRwt patients without carrying the driver genes; (E) PFS in DDRmut patients carrying the driver genes; (F) PFS in all DDRwt patients carrying the driver genes. IPC, immunotherapy plus platinum-based chemotherapy; PC, platinum-based chemotherapy; PFS, progression-free survival; DDRmut, DNA damage repair mutations; DDRwt, DNA damage repair wild-type.

option for NSCLC treatment due to cost and efficacy limitations (52,53). Currently, IPC is the most widely used immune-related therapy in NSCLC, but its association with DDR has not been reported. This is also the first study to demonstrate an independent association between DDR gene mutations and clinical benefit to IPC in patients with advanced NSCLC, and the results show that DDRmut patients still displayed significantly better clinical outcomes than the DDRwt patients. To further explore the correlation between DDR and curative effect, we divided the DDR pathway into smaller pathways. We discovered that the most pronounced PFS benefit for IPC patients was seen in those with mutations in HRR combined with another pathway. This is similar to the results of Wang *et al.*'s study on the prediction of immune efficacy by mutations in HRR-MMR and HRR-BER pathways, respectively (54).

The main mechanism of the DDR pathway is the timely repair of errors during DNA replication and transcription, such as PARP involved in BER and the *BRCA1/2* gene involved in HRR (47). Platinum compounds exert their cytotoxic effects by forming platinum-DNA adducts that interfere with DNA repair and inhibit transcription (55). Generally, when platinum compounds cause the platinum intrastrand crosslinks that forms on DNA, DDR genes can repair these DNA damages to a certain extent. However, when DDR genes are mutated, the DDR pathway will be blocked, which can promote the apoptosis of tumor cells (56). For example, because *BRCA1/2* play key roles in HRR of DNA double-strand breaks, cancers with *BRCA1/2* alterations, often have a better response to DNA cross-linking agents such as platinum compounds (57). Similarly, in our cohort, alterations in the DDR genes were

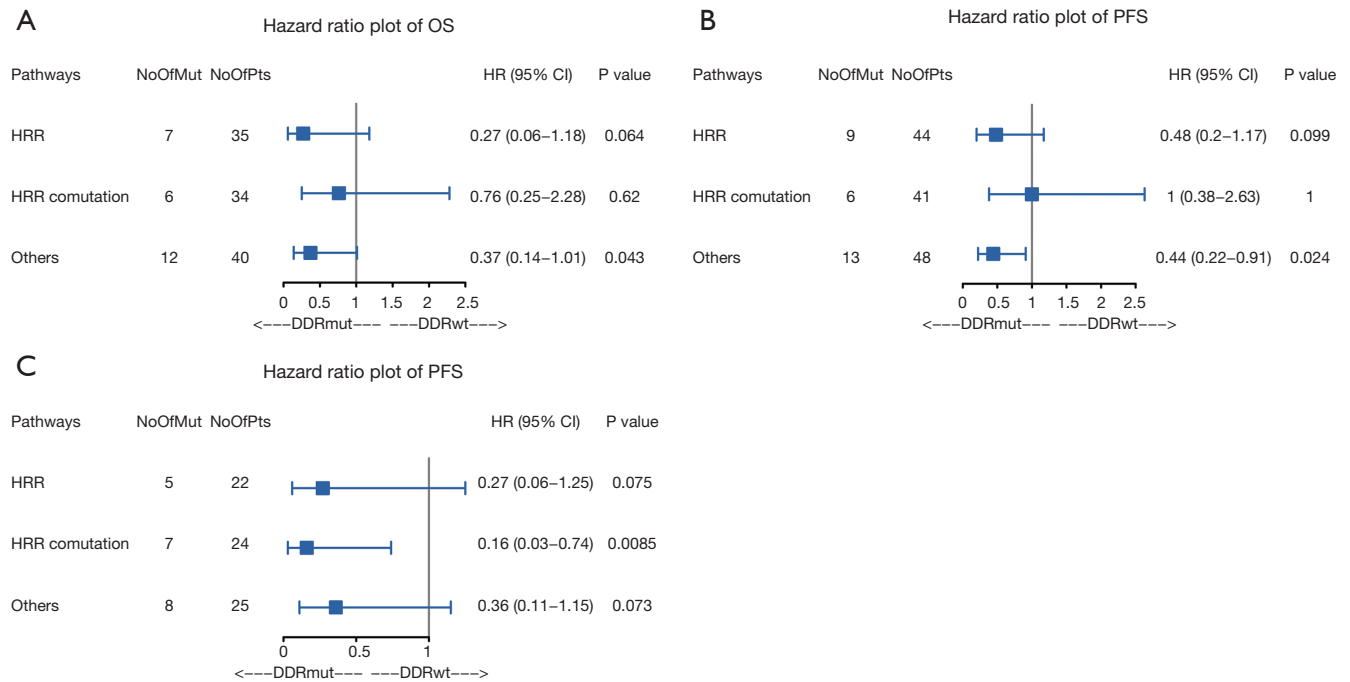

Figure 7 Influence of mutations in different DDR pathways. (A) OS in PC group; (B) PFS in PC group; (C) PFS in IPC group. OS, overall survival; PFS, progression-free survival; HRR, HRR single pathway mutations; HRR comutations, HRR combined with other pathway mutations; others, non-HRR pathway mutations; DDR, DNA damage repair; PC, platinum-based chemotherapy; IPC, immunotherapy plus platinum-based chemotherapy; DDRmut, DNA damage repair mutations; DDRwt, DNA damage repair wild-type; HRR, homologous recombination repair.

significantly associated with better clinical outcomes of PC. In addition, because altering DNA damage responses mediated by exposure to cytotoxic agents or loss of normal DNA repair ability may contribute to antitumor immunity mediated by STING pathways, the DDR mutation may be more sensitive to immunotherapy (25,58-61). When cGAMP synthase (cGAS) interacts with cell-soluble DNA and catalyzes the synthesis of cGAMP, the STING pathway is activated. Activation of STING pathways in antigen-presenting cells (APCs) in tumor microenvironment drives T cells to stimulate tumor-associated antigens and promote the occurrence of anti-tumor immunity (20,62,63). In addition, chemotherapy may promote tumor immunity in two main ways, including inducing immunogenic cell death as part of its intended therapeutic effect and destroying strategies used by tumors to evade immune responses (64). Therefore, the benefits of immunotherapy and synergy of IPC may be more significant for DDRmut patients. However, strictly speaking, the mechanism by which DDR mutation enhances the sensitivity has not been clarified, neither in PC or IPC, and more mechanism studies are needed for elucidation.

This retrospective study had several limitations: (I) the retrospective design of the study and the small sample size of the PC and IPC group may have led to bias in the clinical outcomes observed; (II) more than 200 DDR genes have been reported to date (65), but only 35 were covered by the targeted panel used for this study, so it is reasonable to speculate that some DDR mutations might have been missed during detection; (III) the COSMIC and ClinVar databases are dynamic, and the degree of functional validation that underlies virulence annotations in these databases is variable; (IV) unlike previous research (29), due to the small sample size, our further division of DDR pathway was not sufficiently comprehensive; and (V) since more than half (27/37, 54.05%) of the patients in the IPC group received IPC after second-line treatment (including second-line treatment), by the time the article was submitted, only nine out of 37 patients in the IPC cohort had died, while the remaining 28 patients had not died. In addition, 15 of the 28 patients who did not have a death event were followed for less than 1 year after receiving

IPC, so we believe that the use of current follow-up data to calculate OS is biased.

Conclusions

This study revealed that DDR mutations are common in NSCLC and may predict sensitivity to PC and IPC, especially in the latter. More prospective studies with larger sample sizes are needed to independently verify these findings and allow more robust analyses of individual DDR genes or gene subsets. Further research into the underlying mechanism of the association is also an important priority.

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Footnote

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Data Sharing Statement: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-746/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-746/coif). YZ, MH, and YB are employed by the company 3D Medicines Inc. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Collection and analysis of data were approved by the Ethics Committee of the First Affiliated Hospital of Guangzhou University of Chinese Medicine (No. K-2022-118). The requirement for informed consent was waived because patients, at the time of treatment, consented for their anonymized medical data to be analyzed and published for research purposes.

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Table S1 A panel covering the exons of 381 cancer-related genes

381 cancer-related genes									
ABL1	*ABL2*	*ACVR1B*	*ACVR2A*	*ADAM29*	*ADGRA2*	*AKT1*	*AKT2*	*AKT3*	*ALK*
AMER1	*APC*	*AR*	*ARAF*	*ARFRP1*	*ARID1A*	*ARID1B*	*ARID2*	*ASXL1*	*ATM*
ATR	*ATRX*	*AURKA*	*AURKB*	*AXIN1*	*AXL*	*BAP1*	*BARD1*	*BCL2*	*BCL2L1*
BCL2L11(BIM)	*BCL2L2*	*BCL6*	*BCOR*	*BCORL1*	*BCR*	*BIRC5*	*BLK*	*BLM*	*BMX*
BRAF	*BRCA1*	*BRCA2*	*BRD4*	*BRIP1*	*BTG1*	*BTK*	*C11orf30*	*CARD11*	*CBFB*
CBL	*CCND1*	*CCND2*	*CCND3*	*CCNE1*	*CD274*	*CD79A*	*CD79B*	*CDC73*	*CDH1*
CDK12	*CDK4*	*CDK6*	*CDK8*	*CDKN1A*	*CDKN1B*	*CDKN2A*	*CDKN2B*	*CDKN2C*	*CEBPA*
CHD2	*CHD4*	*CHEK1*	*CHEK2*	*CIC*	*CRBN*	*CREBBP*	*CRKL*	*CRLF2*	*CSF1R*
CSK	*CSNK1A1*	*CTCF*	*CTNNA1*	*CTNNB1*	*CUL3*	*CXCR4*	*CYLD*	*CYP2C19*	*CYP2D6*
DAXX	*DDR1*	*DDR2*	*DICER1*	*DNMT3A*	*DOT1L*	*DPYD*	*EGF*	*EGFR*	*EP300*
EPHA2	*EPHA3*	*EPHA5*	*EPHA7*	*EPHB1*	*ERBB2*	*ERBB3*	*ERBB4*	*ERCC1*	*ERG*
ERRFI1	*ESR1*	*ETV1*	*ETV4*	*ETV5*	*ETV6*	*EZH2*	*FAM135B*	*FAM46C*	*FANCA*
FANCC	*FANCD2*	*FANCE*	*FANCF*	*FANCG*	*FANCL*	*FAS*	*FAT1*	*FBXW7*	*FGF10*
FGF14	*FGF19*	*FGF23*	*FGF3*	*FGF4*	*FGF6*	*FGFR1*	*FGFR2*	*FGFR3*	*FGFR4*
FGR	*FH*	*FLCN*	*FLT1*	*FLT3*	*FLT4*	*FOXL2*	*FOXP1*	*FRS2*	*FUBP1*
FYN	*GABRA6*	*GATA1*	*GATA2*	*GATA3*	*GATA4*	*GATA6*	*GID4*	*GLI1*	*GLI2*
GLI3	*GNA11*	*GNA13*	*GNAQ*	*GNAS*	*GRIN2A*	*GRM3*	*GSK3B*	*H3F3A*	*HCK*
HGF	*HNF1A*	*HRAS*	*HSD3B1*	*HSP90AA1*	*IDH1*	*IDH2*	*IGF1R*	*IGF2*	*IKBKE*
IKZF1	*IL7R*	*INHBA*	*INPP4B*	*IRF2*	*IRF4*	*IRS2*	*ITK*	*JAK1*	*JAK2*
JAK3	*JUN*	*KAT6A*	*KDM5A*	*KDM5C*	*KDM6A*	*KDR*	*KEAP1*	*KEL*	*KIT*
KLHL6	*KMT2A*	*KMT2C*	*KMT2D*	*KRAS*	*LCK*	*LIMK1*	*LMO1*	*LRP1*	*LRP1B*
LYN	*LZTR1*	*MAGI2*	*MAP2K1*	*MAP2K2*	*MAP2K4*	*MAP3K1*	*MAP4K5*	*MCL1*	*MDM2*
MDM4	*MED12*	*MEF2B*	*MEN1*	*MET*	*MITF*	*MLH1*	*MPL*	*MRE11A*	*MS4A1*
MSH2	*MSH6*	*MST1R*	*MTOR*	*MUTYH*	*MYB*	*MYC*	*MYCL*	*MYCN*	*MYD88*
NEK11	*NF1*	*NF2*	*NFE2L2*	*NFKBIA*	*NKX2-1*	*NOTCH1*	*NOTCH2*	*NOTCH3*	*NPM1*
NRAS	*NRG1*	*NRG3*	*NSD1*	*NTRK1*	*NTRK2*	*NTRK3*	*NUP93*	*PAK3*	*PALB2*
PARK2	*PAX5*	*PBRM1*	*PDCD1LG2*	*PDGFRA*	*PDGFRB*	*PDK1*	*PIK3C2B*	*PIK3CA*	*PIK3CB*
PIK3CD	*PIK3CG*	*PIK3R1*	*PIK3R2*	*PKD2*	*PLA2G1B*	*PLCG2*	*PMS2*	*POLD1*	*POLE*
PPP2R1A	*PRDM1*	*PREX2*	*PRKAR1A*	*PRKCI*	*PRKDC*	*PRSS8*	*PTCH1*	*PTEN*	*PTK2*
PTK6	*PTPN11*	*QKI*	*RAC1*	*RAD50*	*RAD51*	*RAF1*	*RANBP2*	*RARA*	*RB1*
RBM10	*RET*	*RICTOR*	*RIT1*	*RNF43*	*ROCK1*	*ROCK2*	*ROS1*	*RPTOR*	*RUNX1*
RUNX1T1	*RXRA*	*SDHA*	*SDHB*	*SDHC*	*SDHD*	*SETD2*	*SF3B1*	*SIK1*	*SLIT2*
SMAD2	*SMAD3*	*SMAD4*	*SMARCA2*	*SMARCA4*	*SMARCB1*	*SMO*	*SNCAIP*	*SOCS1*	*SOX10*
SOX2	*SOX9*	*SPEN*	*SPOP*	*SPTA1*	*SRC*	*SRMS*	*STAG2*	*STAT3*	*STAT4*
STK11	*STK24*	*SUFU*	*SYK*	*TAF1*	*TBX3*	*TCF7L2*	*TEK*	*TERT*	*TET2*
TGFBR1	*TGFBR2*	*TIE1*	*TMPRSS2*	*TNFAIP3*	*TNFRSF14*	*TNFSF11*	*TNK2*	*TOP1*	*TOP2A*
TP53	*TPMT*	*TSC1*	*TSC2*	*TSHR*	*TYK2*	*U2AF1*	*UGT1A1*	*VEGFA*	*VHL*
WEE1	*WEE2*	*WISP3*	*WT1*	*XIAP*	*XPO1*	*YES1*	*ZBTB2*	*ZNF217*	*ZNF703*
ZNF750									

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Table S2 Thirty-five genes of DDR pathway

35 DDR pathway genes			
Gene Symbol	Gene Description	Location	Pathway
MRE11A	MRE11 homolog A, double strand break repair nuclease	11q21	Homologous recombination repair (HRR)
RAD50	RAD50 double strand break repair protein	5q31.1	Homologous recombination repair (HRR)
BARD1	BRCA1 associated RING domain 1	2q35	Homologous recombination repair (HRR)
BLM	Bloom syndrome, RecQ helicase-like	15q26.1	Homologous recombination repair (HRR)
BRCA1	BRCA1 associated RING domain 1	2q35	Homologous recombination repair (HRR)
BRCA2	breast cancer 2, early onset	13q13.1	Homologous recombination repair (HRR)
BRIP1	BRCA1 interacting protein C-terminal helicase 1	17q23.2	Homologous recombination repair (HRR)
PALB2	partner and localizer of BRCA2	16p12.2	Homologous recombination repair (HRR)
MLH1	mutL homolog 1	3p22.2	Mismatch repair (MMR)
MSH2	mutS homolog 2	2p21-p16.3	Mismatch repair (MMR)
MSH6	mutS homolog 6	2p16.3	Mismatch repair (MMR)
PMS2	PMS1 homolog 2, mismatch repair system component	7p22.1	Mismatch repair (MMR)
POLD1	polymerase (DNA directed), delta 1, catalytic subunit	19q13.3	Mismatch repair (MMR)
ATM	ATM serine/threonine kinase	11q22.3	Damage sensor (DS)
ATR	ATR serine/threonine kinase	3q23	Damage sensor (DS)
CHEK1	checkpoint kinase 1	11q24.2	Damage sensor (DS)
CHEK2	checkpoint kinase 2	22q12.1	Damage sensor (DS)
FANCA	Fanconi anemia, complementation group A	16q24.3	Fanconi anemia (FA)
FANCC	Fanconi anemia, complementation group C	9q22.32	Fanconi anemia (FA)
FANCD2	FANCD2/FANCI-associated nuclease 1	15q13.3	Fanconi anemia (FA)
FANCG	Fanconi anemia, complementation group G	9p13.3	Fanconi anemia (FA)
FANCE	Fanconi anemia, complementation group E	6p21.31	Fanconi anemia (FA)
FANCF	Fanconi anemia, complementation group F	11p14.3	Fanconi anemia (FA)
FANCL	Fanconi anemia, complementation group L	2p16.1	Fanconi anemia (FA)
RAD51	RAD51 recombinase	15q15.1	Fanconi anemia (FA)
CUL3	cullin 3	2q36.2	Nucleotide excision repair (NER)
ERCC1	excision repair cross-complementation group 1	19q13.32	Nucleotide excision repair (NER)
POLE	polymerase (DNA directed), epsilon, catalytic subunit	12q24.33	Nucleotide excision repair (NER)
MUTYH	mutY DNA glycosylase	1p34.1	Base excision repair (BER)
PRKDC	protein kinase, DNA-activated, catalytic polypeptid	8q11.21	Non-homologous end-joining (NHEJ)
PTEN	phosphatase and tensin homolog	10q23.31	others
SMARCA4	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4	19p13.2	others
ATRX	ATRX, chromatin remodeler	Xq21.1	others
IDH1	isocitrate dehydrogenase (NADP(+)) 1, cytosolic	2q34	others
WEE1	WEE1 G2 checkpoint kinase	11p15.4	others

DDR, DNA damage repair.

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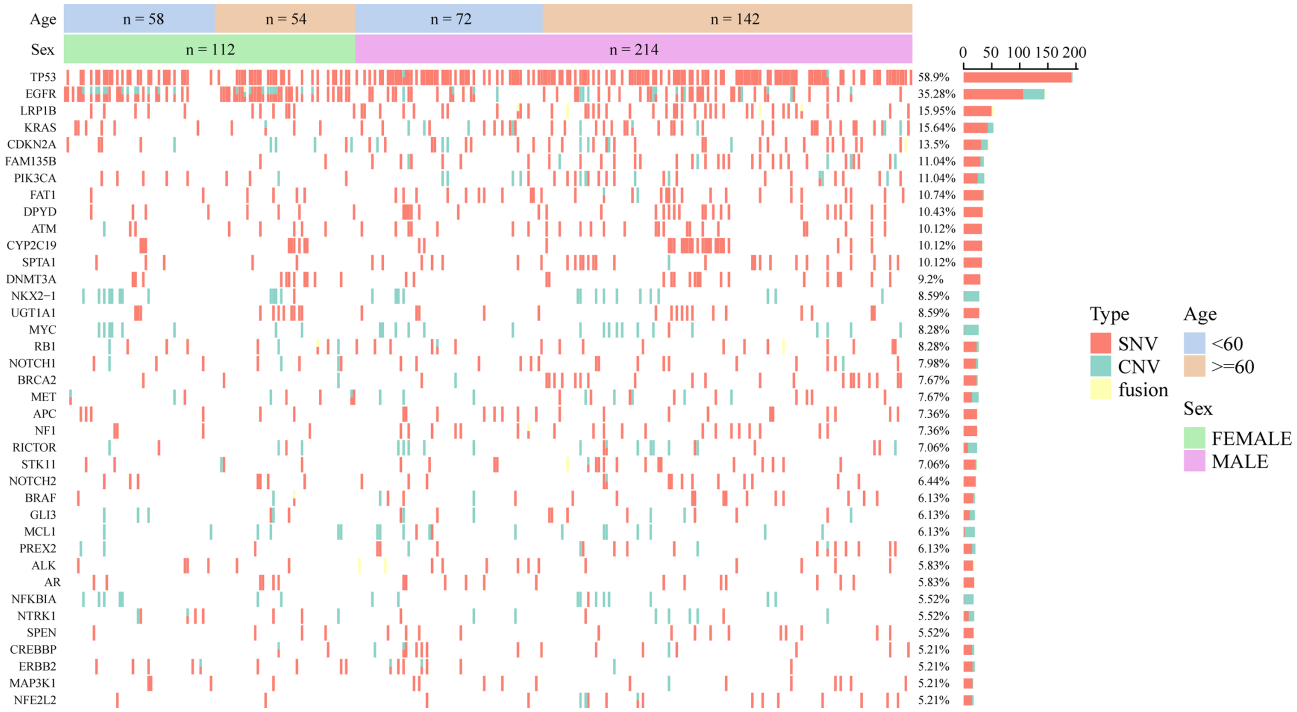

Figure S1 Genomic landscape of total 326 NSCLC patients. NSCLC, non-small cell lung cancer. SNV, single nucleotide variant; CNV, copy number variation.

Figure S2 DDR genomic landscape of NSCLC patients in the DDRmut group. NSCLC, non-small cell lung cancer; DDRmut, DNA damage repair mutations. SNV, single nucleotide variant; CNV, copy number variation.

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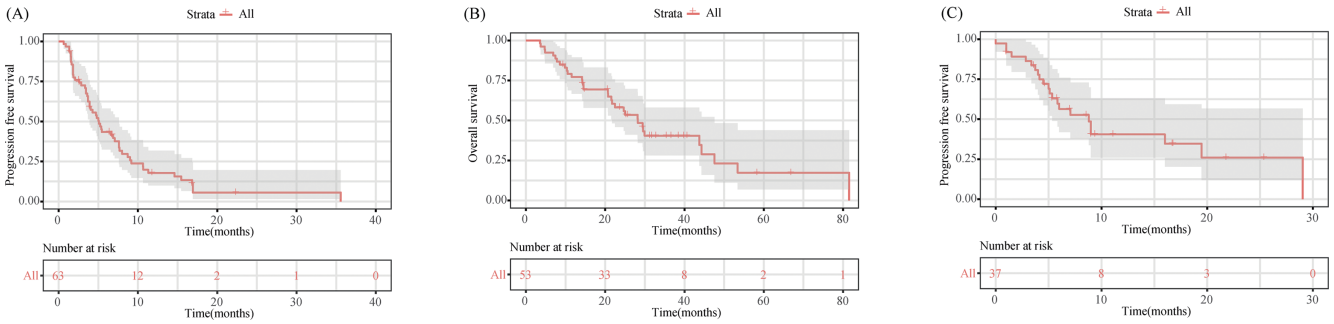

Figure S3 The follow-up time for (A) PFS in PC group, (B) OS in PC group, and (C) PFS in IPC group. PFS, progression-free survival; OS, overall survival; PC, platinum-based chemotherapy; IPC, immunotherapy plus platinum-based chemotherapy.

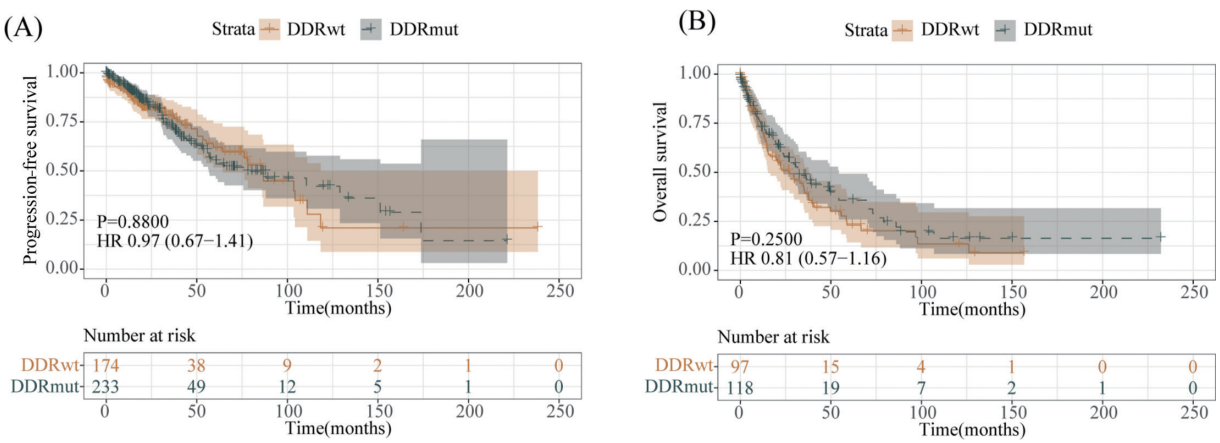

Figure S4 Kaplan-Meier curves of (A) PFS and (B) OS in advanced NSCLC patients of TCGA for DDRmut and DDRwt groups. PFS, progression-free survival; OS, overall survival; DDRmut, DNA damage repair mutations; DDRwt, DNA damage repair wild-type.

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