Lung Cancer 134 (2019) 167–173

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

Homologous recombination and DNA repair mutations in patients treated with carboplatin and *nab*-paclitaxel for metastatic non-small cell lung cancer

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ARTICLE INFO

Keywords:
Non-small cell lung cancer
DNA repair
Homologous recombination
Biomarker
Chemotherapy

ABSTRACT

Objectives: Chemotherapy remains a cornerstone treatment in non-small cell lung cancer either in combination with checkpoint inhibitors or as subsequent therapy. Identifying molecular predictors of response allows for optimal treatment selection. We performed genomic analysis on tumor samples of patients treated with carboplatin and *nab*-paclitaxel as part of a phase II trial to evaluate the prognostic and predictive value of mutations in DNA repair pathway in patients treated with this regimen.

Materials and methods: Next-generation sequencing libraries were produced using a capture-based targeted panel covering the coding exons of 278 genes on patients treated on clinical trial NCT00729612. Overall survival (OS) and progression-free survival (PFS) were assessed as part of the clinical outcomes and correlated with mutation analysis.

Results: Of 63 patients enrolled, 25 patients had sufficient and acceptable DNA isolated from archival tumor samples for targeted sequencing. The most commonly altered pathways included DNA repair (DR) including Fanconi anemia and homologous recombination, JAK-STAT signaling, IGF-1, mTOR, and MAPK-ERK. Four patients with mutations in homologous recombination mutations had a shorter PFS (hazard ratio [HR] = 4.54, 95% CI 1.2, 17.1, p = 0.026) and OS (HR = 6.3, 95% CI 1.8, 21.3, p = 0.003).

Conclusion: In this analysis of patients with predominantly squamous cell non-small cell lung cancer treated with carboplatin and *nab*-paclitaxel in a phase II trial, patients with mutations in homologous recombination pathways had shorter overall and progression-free survival. Validation on additional datasets of patients treated with platinum-based chemotherapy and immunotherapy combinations is warranted.

1. Introduction

Cytotoxic chemotherapy remains an important treatment for patients with non-small cell lung cancer (NSCLC) either alone or in combination with immunotherapy. Studies demonstrating the efficacy of first line treatment with immune checkpoint inhibitors (ICI) alone and in combination with chemotherapy have led to new first-line treatment options for patients with NSCLC [1–3]. Carboplatin, pembrolizumab and either paclitaxel or *nab*-paclitaxel are now standard first line options for patients with lung squamous cell carcinoma (LSCC) [4]. With multiple treatment options now available, patient selection will be key to guide clinical decision making.

A better understanding of the genomic landscape of NSCLC has led to novel treatments for patients with select targetable mutations (i.e. EGFR, ALK, ROS, and BRAF among others), and in many cases targeted therapy offers improved response rates compared to chemotherapy [5–7]. Targeted therapies have so far shown clinical benefit primarily in patients with lung adenocarcinoma where driver mutations are more

Abbreviations: AUC, area under the curve; DR, DNA repair; ICI, immune-checkpoint inhibitor; LSCC, lung squamous cell carcinoma; NSCLC, non-small cell lung cancer; OS, overall survival; PD-L1, programmed death-ligand 1; PFS, progression free survival; TMB, tumor mutational burden

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https://doi.org/10.1016/j.lungcan.2019.06.017
Received 17 April 2019; Received in revised form 14 June 2019; Accepted 15 June 2019

common than in patients with LSCC [8]. Mutations in *TP53* represent the most common alteration in LSCC; mutations in *CDKN2A*, *PIK3CA*, *KEAP1*, *MLL2*, *NFE2L2*, and *RB1* are also frequently observed [8,9]. Mutations in *MLL2*, *CDH8*, *NFE2L2* or *RB1* have been associated with a worse prognosis for patients with early stage LSCC [9]. Mutations in *NFE2L2* and *KEAP1* have also been associated with resistance to chemotherapy and targeted therapies as well as radiation therapy [10,11]. For patients whose tumors do not harbor actionable driver mutations, platinum-based chemotherapy remains a backbone of treatment with or without ICI [12]. For these patients, PD-L1 and tumor mutational burden (TMB) have been the primary biomarkers to guide therapy [2,13]. However, these have been predominantly studied in patients receiving ICI alone, and not in the context of chemotherapy-ICI combinations. There is an ongoing need to develop predictive biomarkers for chemotherapy to enhance patient selection for novel combination therapies.

The mechanism by which platinum agents exert antineoplastic effect is largely through the creation of stable DNA adducts, leading to DNA damage and cell death [14]. This has led to DNA repair mechanisms being evaluated as a possible predictive biomarker for platinum-based therapy [15,16]. Understanding the role of DNA repair pathway mutations in response to platinum based chemotherapy is vital to determine optimal treatment approaches for patients with NSCLC. In this study we retrospectively performed next-generation DNA-sequencing on NSCLC patients who received first-line chemotherapy with carboplatin and nanoparticle albumin-bound (*nab*)-paclitaxel on an NCCN-sponsored clinical trial to evaluate the predictive value of DNA repair pathway mutations in this patient population.

2. Material and methods

Clinical trial NCT00729612 (NCCN A-08) was a phase II trial of carboplatin and *nab*-paclitaxel in 63 patients with advanced or metastatic NSCLC conducted at The Ohio State University with the support of the National Comprehensive Cancer Network (NCCN) Oncology Research Program. The methods and results of the clinical study have been reported previously [17]. Briefly, treatment consisted of *nab*-paclitaxel (300 mg/m^2 for the first 40 patients; dose reduced to 260 mg/m^2 in the remaining 23 patients) and carboplatin area under the curve (AUC) 6 administered as an intravenous infusion on day 1 of a 21-day cycle. The study was conducted between September 2008 and December 2011. Eligible patients included previously untreated patients with contraindications to bevacizumab therapy, including squamous histology, hemoptysis, thromboembolic disease, requirement for therapeutic anticoagulation, or cavitary lung lesions. Major inclusion criteria included measurable disease, ECOG performance status of 0–2, and adequate organ function. Relevant exclusion criteria included recent major surgery, baseline peripheral neuropathy, or previous chemotherapy. All patients were consented as part of the trial to have their initial diagnostic specimen (i.e pretreatment specimen) evaluated for research purposes. The study was approved by the Institutional Review Board and conducted in accordance with Good Clinical Practice Guidelines.

2.1. DNA sequencing

DNA was purified from archival tumor tissue collected from patients consented to the trial (NCT00729612). Next-generation sequencing libraries were produced using a capture-based targeted panel covering the coding exons of 278 genes and libraries were sequenced to a 215X average depth of coverage [18]. Raw sequence reads were processed and aligned to human reference genome using the Genome Analysis Toolkit (GATK) workflow [19]. Ensembl Variant Effect Predictor (VEP) was used to annotate and determine functional consequences of tumor specific variants [20].

2.2. Statistical analysis

NCT00729612 utilized a 2-stage Simon model. The primary endpoint was response rate measured by RECIST. All clinical data was collected as part of the study including objective response rate (ORR), overall survival (OS) and progression-free survival (PFS). These parameters were correlated with mutation analysis. Mutation pathways were grouped according to prior studies [21,22]. Clinical response to treatment was compared between patients whose best response to treatment was partial response versus those with stable disease or progressive disease as best response per RECIST criteria [23]. Kaplan–Meier method and log-rank tests were used to estimate and test the differences of probabilities in OS and PFS between groups. Hazard ratios were estimated by COX regression model. Analyses were performed for all patients with genomic data (genomic evaluable population, GEP) as well as for only squamous cell carcinoma patients within GEP.

3. Results

A total of 63 patients (mean age: 63 yrs) were accrued to the study, with 54 patients evaluable for response. The majority of patients (n = 48) had squamous cell NSCLC, 42 were male (66.7%), and 57 (90%) were current or former smokers (Supplemental Table 1). As reported previously, the overall response rate was 38% (24 partial responses and no complete responses), with median PFS of 5 months and median OS of 9.7 months [17].

3.1. Targeted DNA sequencing

Sufficient DNA sequencing data was available for 25 patients (the genomic evaluable population, GEP). Two patients came off trial for clinical progression before re-staging scans, and another 3 patients were non-evaluable for RECIST response but had clinical disease progression. Four (16%) patients had no known pathogenic mutations detected. The most frequent mutations were in *TP53*, occurring in 13 patients (52%). Other mutations detected included *PRKDC* in 4 (16%) patients; *MAP3K1* and *EPHA5* (3 patients each, 12%); *CREBBP*, *FANCM*, *NOTCH3*, *CSF3R*, *PTEN*, *BLM*, *LRP1B*, *FBXW7*, *RAD50* (2 patients each, 8%). The most represented mutated pathways included DNA repair (DR) pathways such as the Fanconi anemia and homologous recombination repair pathways, JAK-STAT signaling pathway, IGF-1 pathway, and MAPK-ERK pathway (Table 1 and Supplemental Table 2).

3.2. Survival analysis by alterations in non-DNA repair related pathways

When evaluating gene mutations by pathway (Table 1), no pathway alterations were significantly associated with either OS or PFS. For example, 11 patients' tumors with MAPK-ERK pathway mutations had median OS 17.7 compared to median OS 9.6 months for 14 patients' tumors without MAPK-ERK mutations (HR 0.69, 95% CI 0.3, 1.6, p = 0.378). Five patients whose tumors had mutations in IGF-1 pathway had median OS of 14.6 months compared to 13.2 months in 20 those without IGF-1 mutations (HR 0.72, 95% CI 0.24, 2.2, p = 0.5635). Eleven patients with tumor mutations in JAK-STAT pathway had median OS 14.6 months compared to median 13.2 months for 14 of those without STAT mutations (HR 0.8, 95% CI 0.35, 1.8, p = 0.609). Furthermore, alterations in JAK-STAT, IGF-1, or MAPK-ERK were not associated with significant differences in response rates.

3.3. DNA repair pathway mutations

In addition to *TP53* which is important in mediating DNA repair and genomic integrity, other mutations in DNA repair genes detected included *ATR, BLM, BRCA2, ERCC2, FANCA, FANCM, MSH6, PRKDC,* and *RAD50* (Table 1). Interestingly, patients whose tumors harbored DNA

Table 1
Partial response rates by mutation pathways among 23 evaluable patients. Fewer partial responses were observed in patients with DNA repair pathway mutations compared to those without (11% vs 50%, p = 0.069, Fisher's Exact test).

Pathway	Genes Included	Response rate (n,%) in patients with specified mutations	Response rate (n,%) in patients without specified mutations	P-value
DNA Repair	*ATR, BLM, BRCA2, ERCC2, FANCA, FANCM, MSH6, PRKDC, RAD50*	1/9 (11%)	7/14 (50%)	P = 0.069
JAK-STAT	*BTK, CSF3R, JAK1, JAK2, MAP3K1, MAP3K13, MAP3K5, MAP3K9, NOTCH2, NOTCH3, NOTCH4, RAC1*	2/10 (20%)	6/13 (46%)	P = 0.195
IGF-1	*CHUK, IGF1R, IGF2R, JAK1, JAK2, PIK3C2G, PIK3CA, PIK3CG*	0/4 (0%)	8/19 (42%)	P = 0.154
MAPK-ERK	*EPHA5, EPHA6, EPHB1, EPHB6, ERBB4, FGF7, FGFR4, FLT1, FLT3, GRIN2A, JAK1, JAK2, MAP3K1, MAP3K13, MAP3K5, MAP3K9, RB1, VEGFA*	2/9 (22%)	6/14 (43%)	P = 0.290
TP53		5/11 (45%)	3/12 (25%)	P = 0.929

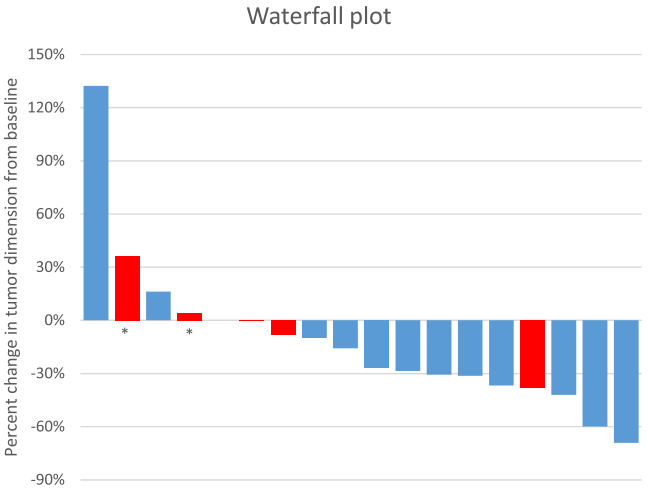

Fig. 1. Waterfall plot: Maximum percent change from baseline in the sum of the diameters of target lesions in 18 evaluable patients with squamous cell histology, with patients' tumors harboring DNA repair mutations present indicated in red. Patients whose tumors possess DNA repair mutations appeared to have less clinical benefit from carboplatin and nab-paclitaxel, although this did not meet statistical significance. Red bars indicate DNA repair mutations are present, asterisk indicates homologous repair mutations.

repair pathway mutations had a lower rate of partial response to treatment by RECIST (11% vs 50%), although this did not meet statistical significance (Table 1, p = 0.069). More specifically, in 18 patients with squamous cell carcinoma evaluable for a response, mutations in the DNA repair pathway (p = 0.382) and homologous recombination pathway (*BLM*, *RAD50*, *BRCA2;* p = 0.446) were not associated with objective response to treatment (Fig. 1). Of the four patients with homologous recombination mutations, there were no partial responses observed, and one patient had clinical progression during the first cycle prior to staging scans and was non-evaluable for radiographic response. The remaining three patients had progressive (n = 1) or stable disease (n = 2) as their best response. Thus, these homologous recombination alterations may be contributing to the trend in differences in response rate between tumors with and without DNA repair pathway mutations (Fig. 1).

Overall, within the GEP, there was no difference in PFS or OS in 10 patients with DNA repair mutations (Fig. 2A, C), and there was no difference in PFS or OS in three patients with mutations in the FA pathway. However, four patients whose tumors bear homologous recombination mutations had significantly shorter PFS than patients without homologous recombination mutations (HR 4.5, 95% CI 1.2, 17.1, p = 0.026, Fig. 2B). In addition, these patients exhibited significantly shorter OS (HR 6.3, 95% CI 1.8, 21.3, p = 0.003, Fig. 2D). Two patients with *RAD50* mutations had significantly worse OS (p = 0.003). For those patients within the GEP with squamous cell histology, homologous recombination mutations remained significantly associated with OS (HR 4.2, 95% CI 1.1, 16.1, p = 0.035, Fig. 3D) although the association with PFS was weaker (HR 4.2, 95% CI 1.0, 17.9, p = 0.05, Fig. 3B). Finally, an analysis of response and survival based on the presence or absence of TP53 alterations revealed no change in response rate (Table 1) or overall survival (Fig. 3).

4. Discussion

Chemotherapy remains a cornerstone of the treatment for patients with advanced NSCLC. For patients with LSCC, treatment with carboplatin and nab-paclitaxel has been shown to have increase rate of response compared to carboplatin and paclitaxel, and improvement in survival has been reported in some patient populations [24]. Recently, the addition of immune-checkpoint inhibitors (ICI) to chemotherapy in

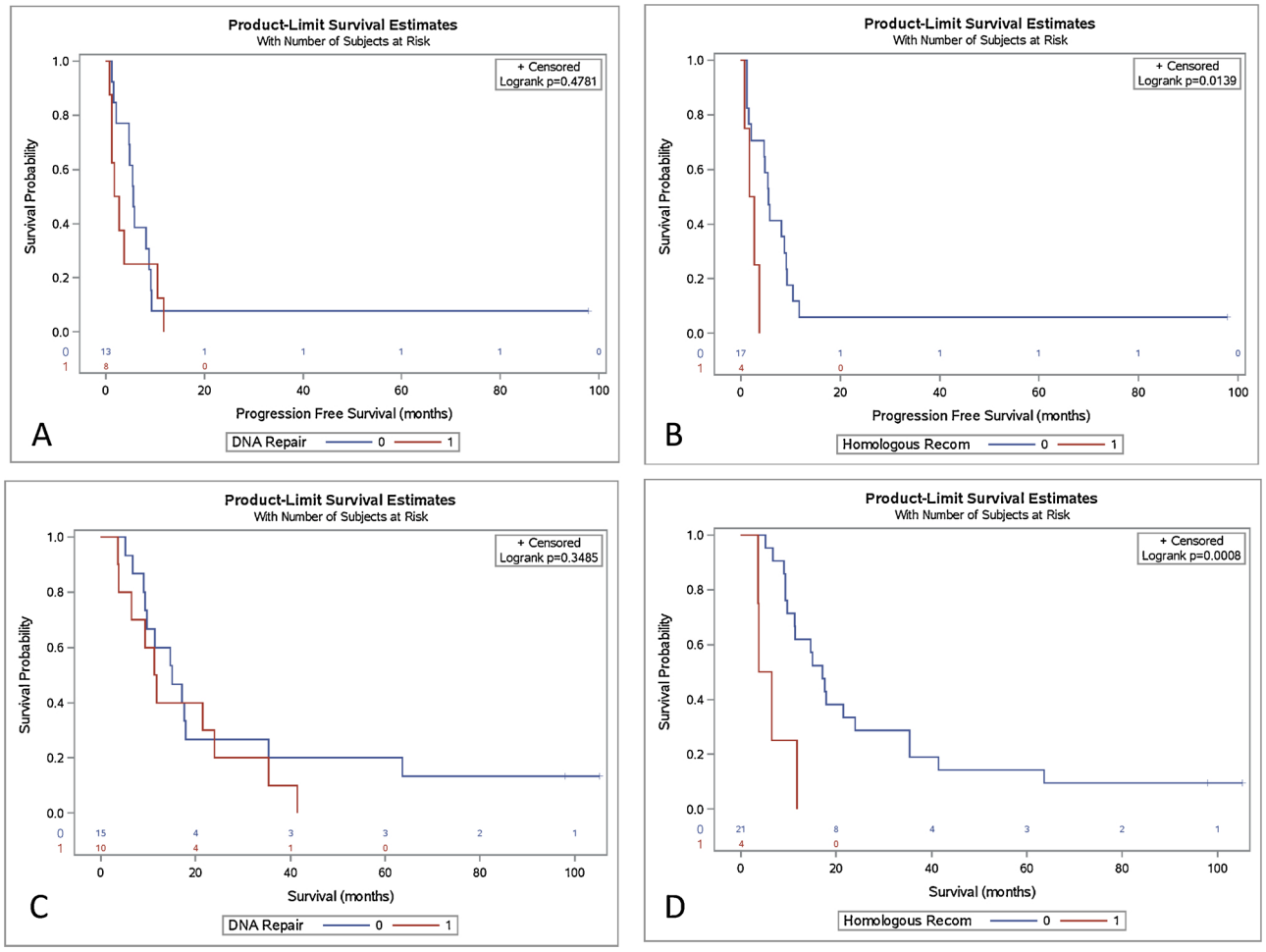

Fig. 2. Kaplan Meier curves for progression free survival (A, B) and overall survival (C, D) within the 25 patient GEP. No statistically significant difference in PFS (A) or OS (C) was observed in patients with tumors possessing mutations in DNA repair pathways. However, patients whose tumors have mutations in the homologous recombination repair pathway had a shorter PFS (B, HR = 4.54, 95% CI 1.2, 17.1, p = 0.026; log-rank p = 0.014), and OS (D, HR = 6.3, 95% CI 1.8, 21.3, p = 0.003; log-rank p = 0.0008).

the first line treatment demonstrated improvement in survival for patients with NSCLC of all histologies compared to chemotherapy alone [1]. Platinum based chemotherapy in combination with ICI is now an approved first line treatment for patients with metastatic NSCLC regardless of histology [1,4]. There remains an urgent need for reliable predictive biomarkers for both chemotherapy and immune-directed therapies. Tumor expression of PD-L1 and TMB have emerged as potential biomarkers for ICI treatment [2,13,25,26]. However, both are imperfect biomarkers due to inter-assay variability and tumor heterogeneity for PD-L1 [27,28] and the lack of standardization for TMB calculation and reporting [13,25]. The role of these biomarkers for patients treated with combination chemotherapy and ICI remains unclear. In this study, we retrospectively evaluated tumor samples from patients treated with carboplatin and nab-paclitaxel in a phase 2 trial to evaluate possible predictive biomarkers. We found that patients whose tumors have mutations in homologous recombination DNA repair pathways had shorter PFS and OS than those without homologous recombination mutations, as well as a trend toward lower response rate. Mutations in other pathways including JAK-STAT, MAPK-ERK, and IGF-1 were not associated with survival or response to treatment.

Aberrations in the genes involved in DNA repair can lead to the development of cancer, and ineffective or defective DNA repair has long been evaluated as a biomarker or target for anti-neoplastic therapy [29]. Mutations leading to defective mismatch repair enzymes results in microsatellite instability which is associated with a response to ICI [30]. In NSCLC, DNA repair pathways have been evaluated as a predictor of clinical benefit from platinum-based chemotherapy. The DNA repair gene excision repair cross-complementation group-1 (ERCC1), a component of the nucleotide excision repair (NER) pathway, has been studied as a predictive biomarker for platinum-based chemotherapy in NSCLC [15,16]. However, encouraging findings of the predictive utility of ERCC1 protein expression were not replicated in subsequent studies, possibly due to differing isoforms of ERCC1 and limitations of the antibodies used for testing [31,32]. DNA repair pathway mutations have been shown to play a role in response to platinum-based chemotherapy in NSCLC including *RAD50* [33,34]. Mutations in DNA repair pathways have also been associated with increased neo-antigen load and T-cell infiltration [21], as well as with overall tumor mutational burden in several cancers [35], including NSCLC [36]. These studies support further investigation of DNA repair pathways as a predictor of response to treatment.

In our study, mutations in homologous recombination genes were associated with poor survival and a trend toward lower response rate. It is well established that mutations in germline homologous recombination genes such as *BRCA1* and *BRCA2* are associated with the development with several cancers including most notably breast and ovarian cancer [37,38]. Treatment with polyadenosine diphosphate-ribose (PARP) inhibitors is associated with dramatic clinical benefit in both breast [39] and ovarian cancer [40], and several PARP inhibitors are now approved for select patients with these mutations. *RAD50* is a key component of the Mre11/Rad50/Nbs1 (MRN) complex which is required for recognition of double-strand breaks, and has also been

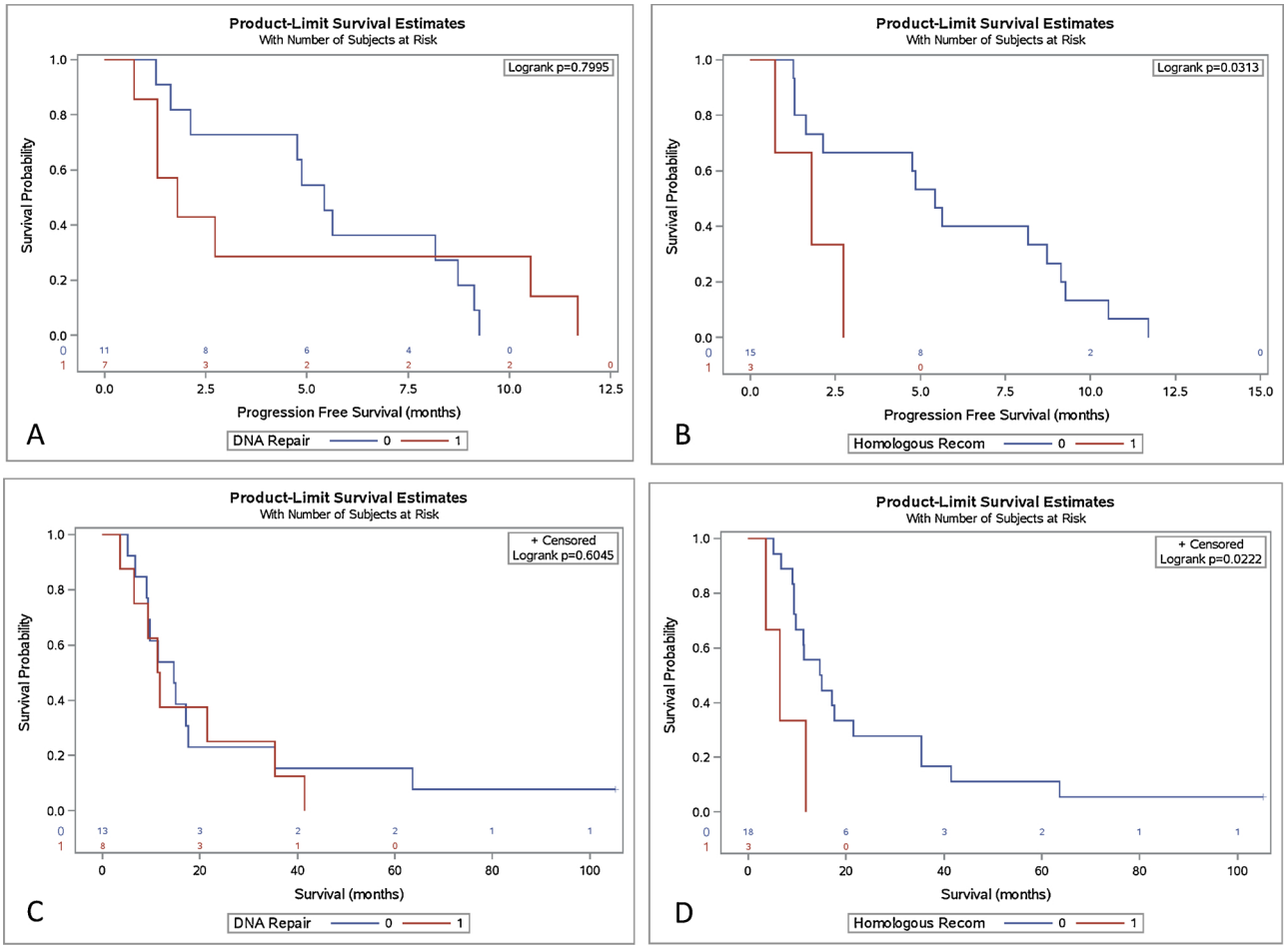

Fig. 3. Kaplan Meier curves for progression free survival (A, B) and overall survival (C, D) for only those patients within the GEP with squamous cell histology. No statistically significant difference in PFS (A) or OS (C) was observed in patients with tumors possessing mutations in DNA repair pathways. However, patients whose tumors have mutations in the homologous recombination repair pathway had a shorter OS (D, HR 4.2, 95% CI 1.1, 16.1, p = 0.035; log-rank p = 0.022) although the association with PFS was weaker (B, HR 4.2, 95% CI 1.0, 17.9, p = 0.05, log-rank p = 0.031).

shown to be involved in cell cycle regulation [41].

Expression of RAD50 protein has also been associated with resistance to radiotherapy in patients with NSCLC [42]. Disruption of the MRN complex due to mutant RAD50 led to increased tumor sensitivity to cisplatin in a murine squamous cell xenograft model [33]. RAD51 protein expression has also been shown to be associated with a worse prognosis in NSCLC [43]. In our study, the two *RAD50* mutations identified included R1198G and E605Q. To our knowledge, the association between mutations in HR repair genes including *RAD50* being associated with decreased response to treatment, PFS, and OS with carboplatin and *nab*-paclitaxel combination therapy in patients with NSCLC has not previously been reported.

The rationale for why mutations in homologous recombination genes are associated with worse outcomes is not clear. Most mutations observed in these genes are predicted to be disruptive, leading to loss of function. Thus, decreased tumor cell DNA repair capacity could be hypothesized to lead to increased tumor cell death and better response to DNA damaging therapy with platinum-based combination chemotherapy. On the other hand, defects in homologous recombination might lead to compensatory activation of other repair pathways such as non-homologous end-joining or nucleotide excision repair that ultimately lead to tumors that are “primed” for DNA repair in the face of DNA damaging chemotherapy. Unfortunately, the activation of these repair pathways (and repair capacity) is not able to be reliably measured by DNA sequencing, and are best assessed using functional DNA repair assays in tumor cell lines or fresh tumor tissue. In addition, while activation can sometimes be inferred by immunohistochemistry of phosphorylated DNA repair proteins, sufficient archival tissue did not exist to perform these studies.

There are several important limitations in our study, including the small sample size and the lack of sufficient tissue to perform DNA sequencing on many of the patients enrolled in the clinical trial. The filtering of genetic calls by likely deleterious impact and mutation allelic frequency may also have led to the omission of possibly relevant mutations. These findings in a subset of patients treated with carboplatin and *nab*-paclitaxel combination therapy may be used to guide studies of patients treated with this regimen in combination with ICI. Lastly, the fact that we observed differences in PFS and OS but not response rate in patients with homologous recombination mutations raises the possibility of these mutations being prognostic but not specifically predictive of response to platinum-based treatment. OS may also be impacted by second and third line treatments which may limit the generalizability of these findings.

5. Conclusion

In the era of first line chemo-immunotherapy for patients with metastatic NSCLC, predictive and reliable biomarkers for response to treatment are more important than ever. In a clinical study of patients with metastatic NSCLC treated with first-line carboplatin and *nab*-paclitaxel with predominantly squamous cell histology, patients with mutations in homologous recombination mutations had shorter overall

and progression-free survival. Patients with mutations in DNA repair genes had a trend toward lower response rate. These findings should be evaluated in other cohorts of patients treated with platinum combination chemotherapy and patients treated with combination chemo-immunotherapy to evaluate the predictive value of these mutations.

Funding

Clinical study NCT00729612 was approved and funded in part by the National Comprehensive Cancer Network (NCCN) Oncology Research Program from general research support provided by Celgene.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.lungcan.2019.06.017.

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