BMJ Open

BMJ Open is committed to open peer review. As part of this commitment we make the peer review history of every article we publish publicly available.

When an article is published we post the peer reviewers' comments and the authors' responses online. We also post the versions of the paper that were used during peer review. These are the versions that the peer review comments apply to.

The versions of the paper that follow are the versions that were submitted during the peer review process. They are not the versions of record or the final published versions. They should not be cited or distributed as the published version of this manuscript.

BMJ Open is an open access journal and the full, final, typeset and author-corrected version of record of the manuscript is available on our site with no access controls, subscription charges or pay-per-view fees (http://bmjopen.bmj.com).

If you have any questions on BMJ Open's open peer review process please email info.bmjopen@bmj.com

BMJ Open

Pembrolizumab Monotherapy for Non-Small Cell Lung Cancer (NSCLC): Can Patient Stratification be Improved in the Tayside Population? A Retrospective Cohort Study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2023-076715
Article Type:	Original research
Date Submitted by the Author:	14-Jun-2023
Complete List of Authors:	Mander, Emily ; University of Dundee, School of Medicine Merrick, Christopher ; NHS Tayside, Tayside Cancer Centre Nicholson, Hugh ; University of Dundee, School of Medicine Lord, Hannah ; NHS Tayside, Tayside Cancer Centre Ferguson, Michelle ; NHS Tayside, Tayside Cancer Centre Smith, Gillian; University of Dundee, School of Medicine
Keywords:	RADIOTHERAPY, CHEMOTHERAPY, Clinical Decision-Making, ONCOLOGY, Respiratory tract tumours < ONCOLOGY

SCHOLARONE™
Manuscripts

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

For peer review only

Manuscript for submission to BMJ Oncology, June 2023

Pembrolizumab Monotherapy for Non-Small Cell Lung Cancer (NSCLC): Can Patient Stratification be Improved in the Tayside Population? A Retrospective Cohort Study

Emily S Mander[1], Christopher B Merrick[2], Hugh A Nicholson[3], Hannah K Lord[2], Michelle J Ferguson[2], Gillian Smith[3]

[1]School of Medicine, University of Dundee; [2]Tayside Cancer Centre; [3]Division of Cellular & Systems Medicine, School of Medicine, University of Dundee, Ninewells Hospital & Medical School, Dundee DD1 9SY

Correspondence to: Dr Gillian Smith, Division of Cellular & Systems Medicine, School of Medicine, University of Dundee, Jacqui Wood Cancer Centre, Ninewells Hospital & Medical School, James Arrott Drive, Dundee DD1 8SY. Email: g.smith@dundee.ac.uk, ORCID 0000-0001-9288-7566.

For peer review only

Abstract

Introduction: Pembrolizumab is a PD-1 inhibitor used to treat advanced NSCLC patients with PD-L1 tumour proportion score (TPS) ≥50%. Further TPS-based stratification has not been evaluated in the UK, although smoking-induced tumour mutational burden and the immunogenic effects of prior radiotherapy are suggested to improve response.

Aims: To investigate if PD-L1 TPS ≥80%, smoking status or radiotherapy before or within 2 months of treatment influenced progression-free survival (PFS) in NSCLC patients treated with pembrolizumab monotherapy.

Methods: PD-L1 TPS, smoking status and radiotherapy exposure were compared in NSCLC patients in NHS Tayside (n=100) treated with pembrolizumab monotherapy between 1st November 2017 and 18th February 2022. Survival estimates were compared using log rank analysis, and Cox proportional hazards analysis used to investigate the influence of potential confounding factors, including tumour stage and performance status.

Results: PFS was not significantly different (log rank hazard ratio (HR)=0.330, p=0.566) comparing patients with PD-L1 TPS 50-79% and PD-L1 TPS ≥80%. Smokers had significantly improved PFS (log rank HR=4.867, p=0.027), while patients receiving radiotherapy had significantly decreased PFS (log rank HR=6.649, p=0.012). A Cox regression model confirmed that both radiotherapy (p=0.022) and performance status (p=0.009) were independent negative predictors of PFS.

Conclusions: More rigorous PD-L1 TPS stratification did not influence survival outcomes. Smoking history improved PFS, although was not an independent response predictor, while radiotherapy and performance status independently influenced clinical response. We suggest that further stratification of PD-L1 TPS is not warranted, while performance status and radiotherapy treatment may be additional clinically useful biomarkers of response to pembrolizumab in NSCLC patients.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Key Messages

What is already known on this topic

- Pembrolizumab is known to improve outcomes in NSCLC patients with PD-L1 TPS ≥50%. Previous studies have described a link between smoking and improved response to pembrolizumab therapy in NSCLC patients. Radiotherapy has been proposed to increase survival in NSCLC patients treated with pembrolizumab, in part due to the acknowledged immunostimulatory effects of radiotherapy.

What this study adds

- This study suggests that further stratification of PD-L1 TPS is not warranted, that the impact of radiotherapy requires further analysis in carefully controlled trials and identifies performance, but not smoking status as an independent predictive biomarker for PFS in NSCLC patients treated with pembrolizumab monotherapy.

How this study might affect research, practice, or policy

- Our findings could influence the way future NSCLC patients are stratified for pembrolizumab monotherapy in routine clinical practice.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Introduction

Lung cancer is the third most common cancer in the UK, and is often diagnosed at late stage, making it the principal cause of cancer mortality in both the UK and the USA (1) (2). Non-small cell lung cancer (NSCLC) comprises the majority of lung cancer cases and encompasses a variety of histological types; adenocarcinoma (40%), squamous cell carcinoma (25%) and large cell carcinoma (10%) (3) (4). Advanced stage NSCLC (TNM stage III and IV) is treated with systemic anticancer therapy (SACT), as surgery is no longer possible (5). Chemotherapy offers poor survival outcomes in patients with advanced NSCLC, with a 1-year survival rate of around 30% (6). While subsets of NSCLCs have actionable targets including epidermal growth factor receptor (EGFR) mutations, anaplastic lymphoma kinase (ALK) translocations, and c-ROS oncogene 1 (ROS-1) rearrangements, the majority of non-small cell lung tumours do not express these oncogenic drivers (7).

Immune checkpoint inhibitors targeting the programmed cell death protein-1/programmed cell death ligand-1 (PD-1/PD-L1) axis have revolutionised the treatment of advanced and metastatic NSCLC, as they provide a stratified treatment option for patients with PD-L1 positive tumours but no other targetable mutations. PD-L1 expression is increased in NSCLC through aberrant signalling mechanisms resulting in T-cell inhibition which allows tumour cells to evade immune destruction (8) (9) (10).

Pembrolizumab is a monoclonal antibody which targets PD-1 on T-cells to disrupt the PD-1/PD-L1 axis (11) (12). Prescription of pembrolizumab in NSCLC is based on PD-L1 tumour proportion score (TPS), the percentage of viable tumour cells expressing PD-L1, assessed by immunohistochemistry as a biomarker to stratify patients (13). In Scotland, Scottish Medicines Consortium (SMC) guidelines approve the use of pembrolizumab as first line monotherapy for advanced NSCLC in patients with PD-L1 TPS ≥50% with no EGFR or ALK mutations. It is also licensed as second line monotherapy for patients with PD-L1 TPS ≥1% who have received at least one prior chemotherapy regime, and as first line treatment in combination with pemetrexed and platinum chemotherapy for advanced NSCLC patients with PD-L1 TPS <50%. Patients must have no other sensitising mutations (e.g., EGFR, ALK, ROS-1) as these can be targeted with other specific inhibitors, such as the EGFR inhibitor gefitinib (14). The Keynote-010 clinical trial investigated superiority of pembrolizumab over docetaxel (OS HR 0·54, 95% CI 0·38–0·77, p=0.002, PFS HR 0·50, 95% CI 0·36–0·70, p=0.0001) (15) and confirmed improved response to pembrolizumab in patients with PD-L1 TPS ≥50%, while the Keynote-042 trial similarly reported improved pembrolizumab outcomes compared with investigator choice chemotherapy, when patients were stratified by TPS ≥50% (OS HR 0·69 (95% CI 0·56–0·85), p=0·0003, PFS HR 0·81 (95% CI 0·67–0·99), p=0·0170) (16).

While pembrolizumab monotherapy is a more effective treatment than chemotherapy for many NSCLC patients, it is associated with significant immune-related adverse effects, including thyroiditis, pneumonitis, colitis, nephritis, hypophysitis, hepatitis, encephalitis, myocarditis and severe cutaneous adverse reactions (SCARs) that can be severe and occasionally life-limiting (15) (17) (18). It is therefore important that the most appropriate patients are selected for pembrolizumab treatment. Disease response to pembrolizumab is

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

routinely evaluated after two to three cycles of therapy and then every six to nine weeks thereafter. Response is evaluated radiologically, usually using CT scans, which are reported using Response Evaluation Criteria in Solid Tumours (RECIST) criteria (7). Pembrolizumab therapy is associated with a rare treatment response known as pseudoprogression, where an initial increase in tumour burden is seen on imaging, with a subsequent reduction resulting in an overall decrease in tumour burden (19). The reported incidence of pseudoprogression in NSCLC patients treated with immune checkpoint inhibitors is only 5% (20), although it is a significant clinical challenge as it is difficult to differentiate from true progression (20).

High mutational burden and associated molecular smoking signatures have been associated with increased efficacy of pembrolizumab therapy (21). Several studies have also linked cigarette smoking to high tumour PD-L1 expression (22) (23) (24) (25). For example, a prospective study in Canada involving 268 advanced NSCLC patients demonstrated that patients with PD-L1 TPS ≥50% who were smokers had a better response to anti-PD-1 immunotherapy than non-smokers. Objective response rate for current smokers was 36% compared to 26% in former smokers and 14% in non-smokers (p=0.02). Overall survival was also significantly increased in smokers compared to non-smokers. At 1-year post-diagnosis, 85.2% of current smokers were alive compared to 56.1% of former smokers and 42.6% of non-smokers (p=0.003) (26).

Radiotherapy can be used to treat NSCLC both palliatively and radically and has been hypothesised to have an immunostimulatory effect (27) (28), resulting from the release of damage-associated molecular pattern molecules (DAMPs) following tumour cell destruction by radiation. DAMPs activate dendritic cells which trigger the immune system to mount a specific T-cell response (29) (30), resulting in an “abscopal effect”, where tumour sites distant from the location of radiotherapy start to regress (31).

A secondary analysis of the Keynote-001 clinical trial of pembrolizumab in NSCLC investigated the effects of radiotherapy prior to pembrolizumab monotherapy and found that patients who had received prior radiotherapy had a significantly increased median progression-free survival of 4.4 months compared to 2.1 months in the group who did not receive prior radiotherapy (p=0.019). At 6 months progression-free survival was 49% in the prior radiotherapy group compared to 23% in patients that did not receive prior radiotherapy (p=0.019). Patients who received radiotherapy prior to pembrolizumab monotherapy also had significantly increased median overall survival of 10.7 months compared to 5.3 months in patients who did not receive prior radiotherapy (p=0.026) (32).

The PEMBRO-RT Phase II clinical trial was designed to investigate whether stereotactic ablative radiotherapy (SABR) prior to pembrolizumab therapy resulted in an enhanced treatment response in metastatic NSCLC, regardless of PD-L1 expression. 76 patients were randomised in a 1:1 ratio to receive either pembrolizumab monotherapy (control group) or SABR prior to pembrolizumab (experimental group). Median progression-free survival was 6.6 months in the SABR group compared to only 1.9 months in the no radiotherapy group, although this difference was not statistically significant (p=0.19) in this relatively small study. Similarly, median overall survival was 15.9 months in the SABR group compared to 7.6 months in the no radiotherapy group (p=0.16) (33).

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

As well as PD-L1 TPS, smoking and radiotherapy there are other important modifiers of outcome to consider for all cancer patients, including the performance status of the patient and the stage and histology of the tumour. Performance status is a measure of the functional status of a patient and is assessed using the Eastern Cooperative Oncology Group Score (ECOG) Performance Status Scale. The score ranges from zero to five, zero indicating no functional deficit and 5 indicating that the patient is deceased (34). Several studies have suggested that patients with performance status ≥2 have worse survival outcomes following pembrolizumab treatment than patients with performance status 0-1 (35) (36) (37).

This study aimed to investigate whether pembrolizumab patient selection could be refined by further sub-division of PD-L1 expression thresholds, and whether previous data describing a positive association of smoking on progression-free survival in NSCLC patients on pembrolizumab therapy was seen in the Tayside population. Based on current literature reporting potential immunostimulatory effects of radiotherapy, we also aimed to investigate the influence of radiotherapy on progression-free survival in NSLCC patients prescribed pembrolizumab in routine clinical practice, out with a controlled clinical trial setting.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Methods

1. Study Approval

Caldicott Guardian Approval was received to allow collection of confidential NSCLC patient information in NHS Tayside.

2. Patient Selection

Study data was collected from NHS computers in Ward 32 Oncology, Ninewells Hospital & Medical School, Dundee between 31st January 2022 and 18th February 2022, with further follow up data collection from 5th January 2023 to 19th February 2023. All patient data was anonymised before inclusion in the study. 150 NSCLC patients were identified from the NHS Tayside oncology database following a diagnosis of non-small cell lung cancer and treatment with at least one cycle of pembrolizumab therapy between November 2017 and February 18th, 2022. Patients were excluded from the study if tumour PD-L1 TPS was unknown or <50%, they refused treatment, died after one cycle of pembrolizumab therapy, or pembrolizumab was prescribed in combination with chemotherapy (triple therapy). Demographic information for all patients, including age, sex, performance status, tumour histology, tumour stage and EGFR, ALK and ROS-1 mutation status was obtained from the Chemocare database, ICE and Clinical Portal.

3. PD-L1 Expression Data

PD-L1 TPS for each tumour, assessed by immunohistochemistry, was obtained from pathology reports or reports from Tayside Lung Cancer Multi-disciplinary Team Meetings (MDTs), obtained from the ICE database. Patients were then stratified into two groups: PD-L1 TPS 50-79% and PD-L1 TPS ≥80%.

4. Radiotherapy Data

Oncology records, accessed through the Clinical Portal database, were used to document the date, type and location of any radiotherapy given. Patients were initially stratified into two groups: those who received radiotherapy at any time before or within two months of immunotherapy, and those who did not receive radiotherapy before or within two months of immunotherapy. Patients were then further sub-divided by palliative or radical radiotherapy, with patients receiving palliative radiotherapy further divided into two subgroups based on radiotherapy location (thoracic or extra thoracic).

5. Smoking Data

Self-reported smoking status was obtained from medical records using the Clinical Portal database. Patients were first divided into two groups: patients who had ever smoked and patients who had never smoked. Patients who had smoked were then further divided into current smokers and former smokers.

6. Study Outcomes

Due to the retrospective nature of the study, many patients went on to receive other forms of systemic anticancer therapy (SACT), so there were many potential confounding variables that could influence overall survival. Therefore, consistent with other similar retrospective cohort studies involving immunotherapy in NSCLC, progression-free survival (PFS) was used

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

as the primary outcome of the study. PFS was calculated as the time in days from the start of cycle one of pembrolizumab therapy to the date of radiological disease progression. Treatment response CT scans were carried out every six to nine weeks in this patient cohort. Overall survival, assessed as a secondary endpoint, was calculated as the time in days between the date of diagnosis and the date of death or census end point (February 18th, 2022).

7. Statistical Analysis

Statistical analysis was carried out using version 27 of the SPSS statistics programme (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0, Armonk, NY: IBM Corp). Progression-free and overall survival were assessed using Log-Rank analysis, with Kaplan-Meier Survival Plots created using the ggplot2 and survival packages and Cairo function in the open-source R programming environment Version 2023.03.1+446 (38). If the Kaplan-Meier Plots produced significant results, further Cox proportional hazards models were constructed in SPSS to investigate whether significant conclusions were influenced by potential confounding variables, including performance status, stage and histology.

8. Patient and Public Involvement Statement

Patients or the public were not involved in the design, conduct, reporting or dissemination plans of our research.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Results

1. Patient Demographics

150 patients were initially assessed for inclusion in the study, however final analysis was carried out on 100 patients as 50 patients did not meet the inclusion criteria - 1 patient refused treatment, 9 patients did not have a sample available for PD-L1 testing, PD-L1 TPS was not quantified in 1 patient, 23 patients received triple therapy, 9 patients had PD-L1 TPS <50% and 7 patients died after one cycle of pembrolizumab (Figure 1). Patient demographics are further summarised in Supplemental Material, Table 1.

2. Does PD-L1 TPS 50-79% in comparison to ≥80% influence PFS or OS?

To investigate if stratification of NSCLC patients for pembrolizumab treatment could be further refined by very high PD-L1 TPS (≥80%), patients were separated into two groups; PD-L1 TPS 50-79% and PD-L1 TPS ≥80%, with PD-L1 TPS assessed as described in Methods. There was no significant difference comparing progression-free survival in NSCLC patients with PD-L1 TPS 50-79% and those with PD-L1 TPS ≥80% (HR=0.330, p=0.566) (Figure 2). Similarly, there was no significant difference in overall survival comparing patients with PD-L1 TPS 50-79% and those with PD-L1 TPS ≥80% (HR=0.120, p=0.729) (Supplemental Material, Figure 1A).

3. Does smoking history influence survival outcomes in NSCLC patients prescribed pembrolizumab?

To investigate if smoking status had a significant impact on PFS, patients were sub-divided according to smoking status, as described in Methods. Patients who were smokers (defined as current or former smokers) had significantly longer progression-free survival compared to patients who were non-smokers (HR=4.867, p=0.027) (Figure 3A). Patients were then further subdivided into current smokers, former smokers and non-smokers, with no significant differences in PFS in current smokers and former smokers (HR=5.248, p=0.073) (Figure 3B). In contrast, no significant difference in overall survival was seen in patients who were smokers and those who were non-smokers (HR=0.288, p=0.591) (Supplemental Material, Figure 1B).

4. Does prior radiotherapy treatment influence survival outcomes in NSCLC patients prescribed pembrolizumab?

To investigate the influence of radiotherapy on progression-free survival, patients were categorised based on whether or not they had received radiotherapy before or within two months of pembrolizumab monotherapy, as described in Methods. In contrast to published data, patients who received radiotherapy had significantly decreased progression-free survival compared to patients who did not receive radiotherapy (HR=6.254, p=0.012) (Figure 4). Similar to our smoking data, there was no significant difference in overall survival between patients who received radiotherapy before or within two months of pembrolizumab monotherapy and those who did not (HR=1.316, p=0.251) (Supplemental Material, Figure 1C).

A Cox Regression Model was then used to investigate whether the significant smoking and radiotherapy associations reported above were modified by potential confounding factors

For peer review only

including performance status, tumour stage and histology. Cox regression analysis confirmed that radiotherapy at any point before or within two months of pembrolizumab monotherapy (p=0.022) and performance status (0.009), but not stage (p=0.126), histology (p=0.827), PD-L1 TPS (p=0.568) or smoking status (p=0.081) were independent predictors of PFS in NSCLC patients treated with pembrolizumab.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Discussion

Approval of pembrolizumab has revolutionised the treatment of advanced and metastatic NSCLC, although treatment is expensive and patient selection limited to immunohistochemical assessment of tumour proportion score (TPS), with patients with PD-L1 TPS ≥50% currently eligible for treatment. To investigate whether more rigorous TPS stratification might influence treatment response in routine clinical practice, we compared PD-L1 TPS 50-79% and PD-L1 TPS ≥80% in a cohort of unselected NSCLC patients, and further investigated whether clinical outcomes were influenced by smoking, previous radiotherapy exposure or could simply be predicted by performance status.

We first investigated whether further stratification of PD-L1 TPS might lead to improved clinical outcomes in NSCLC patients. For consistency with previous reports, we used PFS as our primary and OS as secondary analysis endpoint in order to limit additional sources of variation, as many patients received additional SACT following disease progression on pembrolizumab monotherapy. We found no significant difference in either progression-free (HR=0.330, p=0.566) or overall (HR=0.120, p=0.729) survival, comparing patients with PD-L1 TPS 50-79% and PD-L1 TPS ≥80%, suggesting that further TPS-based patient stratification is not warranted. Our data contrasts with the results of an American retrospective study (n=187 patients), which reported an association of PD-L1 TPS ≥90% with significantly improved PFS (14.5 months vs 4.1 months, HR=0.50, p<0.01) (39). However, similar to our own data, a retrospective cohort study in Japan (n=149 patients), comparing PFS in patients with PD-L1 TPS 50-89% and 90-100% reported no significant difference in progression-free survival (HR=0.78, p=0.34). PFS in the Japanese study at 120 days was 64.4% in PD-L1 TPS 50-89% patients and 63.0% in PD-L1 TPS 90-100% patients (HR=1.03, p=0.09) (40), similar to our own data which reports PFS of 70% at 120 days in the PD-L1 50-79% group and 76% in the PD-L1 ≥80% group (p=0.566). Both the American and Japanese studies used higher (≥90%) PD-L1 TPS to stratify patients, and it is important to note that the American study reported TPS using four different antibodies due to differences in practice between institutions. This observation highlights the limitations of PD-L1 as a quantitative biomarker. Although testing is standardised across Scotland, using the same Dako 22C3 antibody reported in the early Keynote trials (41) (17), PD-L1 TPS is routinely reported following expert pathologist assessment of immunohistochemical staining, with associated inherent variation between centres and reporting pathologists (42). Tumour heterogeneity at diagnosis is additionally recognised to significantly influence PD-L1 expression (43), and it is likely that expression varies further during disease progression and treatment. Despite these limitations, baseline PD-L1 TPS assessed from the initial diagnostic biopsy is currently routinely used to inform patient selection for immunotherapy.

Our data initially confirmed previous reports (23) (24) (44), suggesting that patients who were current or former smokers had significantly longer PFS than non-smokers (HR=4.867, p=0.027). Importantly, PFS in current smokers and former smokers was not significantly different (HR=5.248, p=0.073), suggesting that any smoking history has the potential to modify pembrolizumab response. Consistent with our data, a recent meta-analysis investigating the impact of smoking status on targeted therapy in NSCLC in Phase III clinical trials reported that smokers had significantly extended PFS following immune checkpoint inhibitor treatment (HR=1.81, p=0.004) (44), with additional meta-analyses reporting similar

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

conclusions (23) (24). It is also important to note, however, that our extended Cox regression analysis did not confirm smoking history as an independent predictor of pembrolizumab response in NSCLC, and that the influence of confounding factors has not always been previously reported. Although it is logical that smoking may increase tumour mutation burden (TMB) and, as a consequence, increase immunogenicity and improve response to immunotherapy, it is important to acknowledge that TMB has not been routinely assessed in significant numbers of patients outwith the clinical trial setting, and that results from some previous studies do not support this hypothesis (21). The use of smoking status as a biomarker for pembrolizumab response additionally raises important ethical issues as smoking cessation is an important part of the clinical management of lung cancer, as it improves outcomes and reduces the risk of the development of further cancers (45) (46) and other diseases associated with smoking such as cardiovascular disease and chronic obstructive pulmonary disease (COPD) (47). Further, in this and previous studies, patients were identified as smokers or non-smokers based on self-reported smoking history. Verification of smoking status, for example using biochemical confirmation of serum cotinine levels, is recommended but is challenging outwith the clinical trial setting (48), and self-reported smoking history is more likely to be under rather over-represented, in turn under-estimating pembrolizumab response predictions in smokers. Serum cotinine has been successfully used to confirm self-reported smoking status to identify eligible patients for lung cancer screening (48) and can also be used in patients using electronic cigarettes containing nicotine (49) (50).

Our analysis suggests that NSCLC patients receiving radiotherapy before or within two months of pembrolizumab monotherapy had significantly decreased PFS compared to patients who did not receive radiotherapy (HR=6.254, p=0.012), in contrast to the findings of the Keynote-001 clinical trial (32) which reported that radiotherapy increased the efficacy of immunotherapy, possibly due to the abscopal effect (51). Further studies, however, including a retrospective multicentre study evaluating the effects of palliative radiotherapy before or within three months of anti-PD-1 therapy reported no significant difference in PFS, comparing patients who had received radiotherapy and those who had not (3.2 months vs 2.0 months, p=0.515) (52), while the PEMBRO-RT trial also reported no significant difference in PFS in patients who received SABR prior to pembrolizumab therapy and those who did not (1.9 months vs 6.6 months, p=0.19) (53). We acknowledge that patients receiving radiotherapy within 2 months of pembrolizumab in our study may have had more advanced disease, or may have progressed more quickly, although tumour stage at diagnosis was not independently predictive of PFS.

In contrast to previously reported clinical trial data, the majority of patients in the current study received palliative radiotherapy (usually 8Gy in 1 fraction or 20Gy in 5 fractions (54)) rather than SABR. It is therefore possible that palliative radiotherapy does not potentiate immunogenicity in NSCLC patients, as most previous literature reports on the influence of higher dose SABR on immunotherapy outcomes (55). As many of our study patients had symptomatic metastases, it is also possible that the modifying effect of radiotherapy we report, while independently predictive of survival outcomes, may simply represent a surrogate marker for performance status. Many NSCLC patients are additionally prescribed steroids, either to alleviate tumour compression or the side effects of immunotherapy. Steroid use is known to suppress the immune system and may therefore further modify responses to both radiotherapy and immunotherapy (56). We highlight the need to

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

investigate the potential modifying effect of steroid prescription in future studies, and also the potential modifying effect of radiotherapy and pembrolizumab scheduling, as tumour repopulation post radiotherapy may further influence pembrolizumab response (57) (58). It is also important to ensure that CT scan reporting is standardised as far as is practicable in routine clinical practice. In the Keynote-024 clinical trial, for example, CT scans were all reported according to RECIST criteria, by a radiologist independent from the trial (8). While undoubtedly increasing the accuracy of clinical response estimates, greater variation in CT reporting in routine clinical practice is inevitable, even in a single centre. Radiological response assessment is particularly important following immunotherapy treatment due to pseudo-progression, where an initial apparent increase in tumour burden due to accumulation of immune cells causing an inflammatory response result in enlargement of neoplastic lesions (19), followed by subsequent regression (59), and is difficult to differentiate from true disease progression through initial imaging (20) (60). To address this relatively rare complication (incidence <6% in NSCLC patients), revised Response Evaluation Criteria in Solid Tumours (RECIST) guidelines, iRECIST, were developed in 2017 to improve reporting in immunotherapy clinical trials (61).

Importantly, despite these acknowledged sources of variation in biomarker and radiological assessment, our data highlights that performance status is an independent predictor of PFS (p=0.009). We assessed outcomes in all NSCLC patients treated with pembrolizumab (performance status 0-3), in contrast to more restricted clinical trials where, for example, only patients with performance status 0-1 were included in the Keynote-024 clinical trial (17), and the PePS2 single arm Phase 2 trial evaluated pembrolizumab response in patients with PS ≥2 (62). Consistent with our findings, several previous studies have reported that patients with PS ≥2 have reduced survival outcomes (35) (36) (37), while a recent Italian multicentre retrospective study confirmed that performance status was an independent predictor of poor clinical outcome (63).

In conclusion, therefore, our data confirms that more rigorous stratification of NSCLC patients by PD-L1 TPS did not influence survival outcomes. Smoking status (current or previous smoker) significantly improved PFS, although was not an independent predictor of survival. In contrast, radiotherapy treatment at any point before or within two months of pembrolizumab therapy independently adversely influenced PFS, and performance status was shown to be an independent predictor of clinical response. We suggest that further stratification of PD-L1 TPS is not warranted, the modifying effects of radiotherapy require further investigation in carefully controlled future studies, and performance status in addition to the currently used PD-L1 TPS ≥50% may be a clinically useful biomarker of response to pembrolizumab in NSCLC patients.

For Peer Review Only

Figure Legends

Figure 1: Patient Selection and Demographics
150 NSCLC patients were initially identified in NHS Tayside, between 31st January 2022 and 18th February 2022. 50 patients were excluded from the study as they failed to meet the inclusion criteria, for the reasons indicated. Patients were classified as smokers if they were current or ex-smokers, based on self-reported smoking status.

Figure 2: Further patient stratification by PD-L1 TPS does not influence PFS
Log-Rank analysis, represented as Kaplan-Meier survival plots was used to compare PFS in NSCLC patients with PD-L1 TPS ≥80% (red) and PD-L1 TPS 50-79% (blue).

Figure 3: Smoking history influences PFS in NSCLC patients prescribed pembrolizumab
Log-Rank analysis, represented as Kaplan-Meier survival plots was used to compare PFS in (A) smokers (former and current; red) and non-smokers (blue) and in (B) current smokers (red), former smokers (green), and non-smokers (blue).

Figure 4: Prior Radiotherapy influences PFS in NSCLC patients prescribed pembrolizumab
Log-Rank analysis, represented as Kaplan-Meier survival plots was used to compare PFS in NSCLC patients who received radiotherapy before or within two months of pembrolizumab (red) and those who did not receive radiotherapy in that time frame (blue).

Supplemental Material:

Supplemental Material, Figure 1A: Further patient stratification by PD-L1 TPS does not influence OS in NSCLC patients
Log-Rank analysis, represented as Kaplan-Meier survival plots was used to compare OS in NSCLC patients with PD-L1 TPS ≥80% (red) and PD-L1 TPS 50-79% (blue).

Supplemental Material, Figure 1B: Smoking history did not influence OS in NSCLC patients prescribed pembrolizumab
Log-Rank analysis, represented as Kaplan-Meier survival plots was used to compare OS in (A) smokers (former and current; red) and non-smokers (blue).

Supplemental Material, Figure 1C: Prior Radiotherapy did not influence OS in NSCLC patients prescribed pembrolizumab
Log-Rank analysis, represented as Kaplan-Meier survival plots was used to compare OS in NSCLC patients who received radiotherapy before or within two months of pembrolizumab (red) and those who did not receive radiotherapy in that time frame (blue).

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

References:

1. Cancer Research UK. Lung Cancer Statistics, 2018 [Available from: https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/lung-cancer.]
2. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. CA Cancer J Clin. 2022;72(1):7-33.
3. NHS. Lung Cancer, 2019 [Available from: https://www.nhs.uk/conditions/lung-cancer/.]
4. National Cancer Institue. Non-Small Cell Lung Cancer Treatment: National Cancer Institute; 2022 [Available from: https://www.cancer.gov/types/lung/hp/non-small-cell-lung-treatment-pdq#_359.]
5. Molina JR, Yang P, Cassivi SD, Schild SE, Adjei AA. Non-Small Cell Lung Cancer: Epidemiology, Risk Factors, Treatment, and Survivorship. Mayo Clin Proc. 2008;83(5):584-94.
6. Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, et al. Comparison of Four Chemotherapy Regimens for Advanced Non–Small-Cell Lung Cancer. New England Journal of Medicine. 2002;346(2):92-8.
7. Planchard D, Popat S, Kerr K, Novello S, Smit EF, Faivre-Finn C, et al. Metastatic Non-Small Cell Lung Cancer: ESMO Clinical Practice Guidelines for Diagnosis, Treatment and Follow-up. Annals of Oncology. 2018;29(Supplement_4):iv192-iv237.
8. Ghosh C, Luong G, Sun Y. A Snapshot of the PD-1/PD-L1 Pathway. Journal of Cancer. 2021;12(9):2735-46.
9. Jiang Y, Chen M, Nie H, Yuan Y. PD-1 and PD-L1 in Cancer Immunotherapy: Clinical Implications and Future Considerations. Human Vaccines & Immunotherapeutics. 2019;15(5):1111-22.
10. Hanahan D, Weinberg RA. Hallmarks of Cancer: The Next Generation. Cell. 2011;144(5):646-74.
11. Kwok G, Yau TC, Chiu JW, Tse E, Kwong YL. Pembrolizumab (Keytruda). Hum Vaccin Immunother. 2016;12(11):2777-89.
12. BNF. Pembrolizumab | Drug | BNF content published by NICE: NICE; [Available from: https://bnf.nice.org.uk/drug/pembrolizumab.html.]
13. De Marchi P, Leal LF, Duval da Silva V, da Silva ECA, Cordeiro de Lima VC, Reis RM. PD-L1 Expression by Tumor Proportion Score (TPS) and Combined Positive Score (CPS) are Similar in Non-Small Cell Lung Cancer (NSCLC). Journal of Clinical Pathology. 2021;74(11):735-40.
14. SMC. Pembrolizumab (Keytruda): Scottish Medicines Consortium; [Available from: https://www.scottishmedicines.org.uk/medicines-advice/pembrolizumab-keytruda-fullsubmission-123917/.]
15. Herbst RS, Baas P, Kim DW, Felip E, Pérez-Gracia JL, Han JY, et al. Pembrolizumab Versus Docetaxel for Previously Treated, PD-L1-Positive, Advanced Non-Small-Cell Lung Cancer (KEYNOTE-010): A Randomised Controlled trial. Lancet. 2016;387(10027):1540-50.
16. Gandhi L, Rodríguez-Abreu D, Gadgeel S, Esteban E, Felip E, De Angelis F, et al. Pembrolizumab Plus Chemotherapy in Metastatic Non-Small-Cell Lung Cancer. N Engl J Med. 2018;378(22):2078-92.
17. Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, et al. Pembrolizumab Versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. N Engl J Med. 2016;375(19):1823-33.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

18. EMA. Keytruda - Summary of Product Characteristics [Available from: https://www.ema.europa.eu/en/documents/product-information/keytruda-epar-product-information_en.pdf.]
19. Jia W, Gao Q, Han A, Zhu H, Yu J. The Potential Mechanism, Recognition and Clinical Significance of Tumor Pseudoprogression after Immunotherapy. Cancer Biol Med. 2019;16(4):655-70.
20. Park HJ, Kim KW, Pyo J, Suh CH, Yoon S, Hatabu H, et al. Incidence of Pseudoprogression During Immune Checkpoint Inhibitor Therapy for Solid Tumors: A Systematic Review and Meta-Analysis. Radiology. 2020;297(1):87-96.
21. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Mutational Landscape Determines Sensitivity to PD-1 Blockade in Non-Small Cell Lung Cancer. Science. 2015;348(6230):124-8.
22. Norum J, Nieder C. Tobacco Smoking and Cessation and PD-L1 Inhibitors in Non-Small Cell Lung Cancer (NSCLC): A Review of the Literature. ESMO Open. 2018;3(6):e000406.
23. Zhao W, Jiang W, Wang H, He J, Su C, Yu Q. Impact of Smoking History on Response to Immunotherapy in Non-Small-Cell Lung Cancer: A Systematic Review and Meta-Analysis. Front Oncol. 2021;11:703143.
24. Lee KWC, Lord SJ, Kasherman L, Marschner I, Stockler M, Gralla R, et al. The Impact of Smoking on the Effectiveness of Immune Checkpoint Inhibitors — A Systematic Review and Meta-analysis. Acta Oncologica. 2020;59(1):96-100.
25. Abdel-Rahman O. Smoking and EGFR Status May Predict Outcomes of Advanced NSCLC Treated with PD-(L)1 Inhibitors Beyond First Line: A Meta-analysis. The Clinical Respiratory Journal. 2018;12(5):1809-19.
26. Li JJN, Karim K, Sung M, Le LW, Lau SCM, Sacher A, et al. Tobacco Exposure and Immunotherapy Response in PD-L1 Positive Lung Cancer Patients. Lung Cancer. 2020;150:159-63.
27. SIGN. Management of Lung Cancer. 2014.
28. Reynders K, Illidge T, Siva S, Chang JY, De Ruysscher D. The Abscopal Effect of Local Radiotherapy: Using Immunotherapy to Make a Rare Event Clinically Relevant. Cancer Treatment Reviews. 2015;41(6):503-10.
29. Bhalla N, Brooker R, Brada M. Combining Immunotherapy and Radiotherapy in Lung Cancer. Journal of Thoracic Disease. 2018;10(S13):S1447-S60.
30. Corke L, Sacher A. New Strategies and Combinations to Improve Outcomes in Immunotherapy in Metastatic Non-Small-Cell Lung Cancer. Current Oncology. 2021;29(1):38-55.
31. Turgeon GA, Weickhardt A, Azad AA, Solomon B, Siva S. Radiotherapy and Immunotherapy: A Synergistic Effect in Cancer Care. Medical Journal of Australia. 2019;210(1):47-53.
32. Shaverdian N, Lisberg AE, Bornazyan K, Veruttipong D, Goldman JW, Formenti SC, et al. Previous Radiotherapy and the Clinical Activity and Toxicity of Pembrolizumab in the Treatment of Non-Small-Cell Lung Cancer: A Secondary Analysis of the KEYNOTE-001 Phase 1 Trial. The Lancet Oncology. 2017;18(7):895-903.
33. Welsh J, Menon H, Chen D, Verma V, Tang C, Altan M, et al. Pembrolizumab With or Without Radiation Therapy for Metastatic Non-Small Cell Lung Cancer: A Randomized Phase I/II Trial. Journal for ImmunoTherapy of Cancer. 2020;8(2):e001001.
34. ECOG-ACRIN Cancer Research Group. ECOG Peformance Status Scale [Available from: https://ecog-acrin.org/resources/ecog-performance-status/.]

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

35. Tamiya M, Tamiya A, Hosoya K, Taniguchi Y, Yokoyama T, Fukuda Y, et al. Efficacy and Safety of Pembrolizumab as First-Line Therapy in Advanced Non-Small Cell Lung Cancer With At Least 50% PD-L1 Positivity: A Multicenter Retrospective Cohort Study (HOPE-001). Investigational new drugs. 2019;37(6):1266-73.
36. Sehgal K, Gill RR, Widick P, Bindal P, McDonald DC, Shea M, et al. Association of Performance Status With Survival in Patients With Advanced Non-Small Cell Lung Cancer Treated With Pembrolizumab Monotherapy. JAMA Netw Open. 2021;4(2):e2037120.
37. Addeo A, Metro G, Signorelli D, Economopoulou P, Roila F, Banna GL, et al. Poor Performance Status and Front-Line Pembrolizumab in Advanced Non-Small-Cell Lung Cancer (NSCLC) Patients with PD-L1>50%. Journal of Clinical Oncology. 2020;38(15_suppl):e21651-e.
38. R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
39. Aguilar EJ, Ricciuti B, Gainor JF, Kehl KL, Kravets S, Dahlberg S, et al. Outcomes to First-Line Pembrolizumab in Patients with Non-Small-Cell Lung Cancer and Very High PD-L1 Expression. Ann Oncol. 2019;30(10):1653-9.
40. Edahiro R, Kanazu M, Kurebe H, Mori M, Fujimoto D, Taniguchi Y, et al. Clinical outcomes in Non-Small Cell Lung Cancer Patients with an Ultra-High Expression of Programmed Death Ligand-1 Treated Using Pembrolizumab As A First-Line Therapy: A Retrospective Multicenter Cohort Study In Japan. PloS one. 2019;14(7):e0220570-e.
41. Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, et al. Pembrolizumab for the Treatment of Non–Small-Cell Lung Cancer. New England Journal of Medicine. 2015;372(21):2018-28.
42. Ming Sound Tsao KMK, Sanja Dacic, Yasushi Yatabe, Fred R. Hirsch. IASLC Atlas of PD-L1 Immunohistochemistry Testing in Lung Cancer: International Association for the Study of Lung Cancer.
43. Munari E, Mariotti FR, Quatrini L, Bertoglio P, Tumino N, Vacca P, et al. PD-1/PD-L1 in Cancer: Pathophysiological, Diagnostic and Therapeutic Aspects. International Journal of Molecular Sciences. 2021;22(10):5123.
44. Li X, Huang C, Xie X, Wu Z, Tian X, Wu Y, et al. The Impact of Smoking Status on the Progression-Free Survival of Non-Small Cell Lung Cancer Patients Receiving Molecularly Target Therapy or Immunotherapy Versus Chemotherapy: A Meta-Analysis. Journal of Clinical Pharmacy and Therapeutics. 2021;46(2):256-66.
45. Luo SJ, Choi E, Aredo JV, Wilkens LR, Tammemägi MC, Le Marchand L, et al. Smoking Cessation After Lung Cancer Diagnosis and the Risk of Second Primary Lung Cancer: The Multiethnic Cohort Study. JNCI Cancer Spectr. 2021;5(5).
46. Caini S, Del Riccio M, Vettori V, Scotti V, Martinoli C, Raimondi S, et al. Quitting Smoking At or Around Diagnosis Improves the Overall Survival of Lung Cancer Patients: A Systematic Review and Meta-Analysis. J Thorac Oncol. 2022;17(5):623-36.
47. NHS. What Are The Health Risks Of Smoking? : NHS; 2018 [Available from: https://www.nhs.uk/common-health-questions/lifestyle/what-are-the-health-risks-of-smoking/.]
48. Liu B, Henschke CI, Flores RM, Taioli E. Serum Cotinine Verification of Self-Reported Smoking Status Among Adults Eligible for Lung Cancer Screening in the 1999-2018 National Health and Nutrition Examination Survey. Lung Cancer. 2020;144:49-56.
49. Vélez de Mendizábal N, Jones DR, Jahn A, Bies RR, Brown JW. Nicotine and Cotinine Exposure from Electronic Cigarettes: A Population Approach. Clin Pharmacokinet. 2015;54(6):615-26.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

50. Rapp JL, Alpert N, Flores RM, Taioli E. Serum Cotinine Levels and Nicotine Addiction Potential of E-Cigarettes: An NHANES Analysis. Carcinogenesis. 2020;41(10):1454-9.
51. Theelen WS, De Jong MC, Baas P. Synergizing Systemic Responses by Combining Immunotherapy with Radiotherapy in Metastatic Non-Small Cell Lung Cancer: The Potential of the Abscopal Effect. Lung Cancer. 2020;142:106-13.
52. Samuel E, Lie G, Balasubramanian A, Hiong A, So Y, Voskoboynik M, et al. Impact of Radiotherapy on the Efficacy and Toxicity of anti-PD-1 Inhibitors in Metastatic NSCLC. Clinical Lung Cancer. 2021;22(3):e425-e30.
53. Theelen WSME, Peulen HMU, Lalezari F, Van Der Noort V, De Vries JF, Aerts JGJV, et al. Effect of Pembrolizumab After Stereotactic Body Radiotherapy vs Pembrolizumab Alone on Tumor Response in Patients With Advanced Non–Small Cell Lung Cancer. JAMA Oncology. 2019;5(9):1276.
54. Lord H. Clinical Management Protocol - Non-Surgical Management of Non-Small Cell Lung Cancer: NHS Tayside; 2015 [Available from: https://www.nhstaysideadtc.scot.nhs.uk/tapg%20html/Specialist%20Lists/Oncology-Haematology/Protocols/Nonsmall%20cell%20lung%20prot.pdf.]
55. Damen PJJ, Verhoeff JJC. Efficacy Of Stereotactic Ablative Radiotherapy (SABR) During Anti-PD-1 In Oligoprogressive Non-Small Cell Lung Cancer And Melanoma-A Prospective Multicenter Observational Study Pointing Out New Unmet Needs. Transl Cancer Res. 2023;12(3):688-91.
56. Petrelli F, Signorelli D, Ghidini M, Ghidini A, Pizzutilo EG, Ruggieri L, et al. Association of Steroids Use With Survival In Patients Treated With Immune Checkpoint Inhibitors: A Systematic Review And Meta-Analysis. Cancers (Basel). 2020;12(3).
57. Ng WL, Huang Q, Liu X, Zimmerman M, Li F, Li CY. Molecular Mechanisms Involved In Tumor Repopulation After Radiotherapy. Transl Cancer Res. 2013;2(5):442-8.
58. Pajonk F, Vlashi E, McBride WH. Radiation Resistance Of Cancer Stem Cells: The 4 R's Of Radiobiology Revisited. Stem Cells. 2010;28(4):639-48.
59. Ma Y, Wang Q, Dong Q, Zhan L, Zhang J. How to Differentiate Pseudoprogression from True Progression in Cancer Patients Treated with Immunotherapy. Am J Cancer Res. 2019;9(8):1546-53.
60. Zhou L, Zhang M, Li R, Xue J, Lu Y. Pseudoprogression and Hyperprogression In Lung Cancer: A Comprehensive Review Of Literature. Journal of Cancer Research and Clinical Oncology. 2020;146(12):3269-79.
61. Seymour L, Bogaerts J, Perrone A, Ford R, Schwartz LH, Mandrekar S, et al. iRECIST: Guidelines For Response Criteria For Use in Trials Testing Immunotherapeutics. Lancet Oncol. 2017;18(3):e143-e52.
62. Middleton G, Brock K, Savage J, Mant R, Summers Y, Connibear J, et al. Pembrolizumab in Patients with Non-Small-Cell Lung Cancer of Performance Status 2 (PePS2): A Single Arm, Phase 2 Trial. The Lancet Respiratory Medicine. 2020;8(9):895-904.
63. Cortellini A, Tiseo M, Banna GL, Cappuzzo F, Aerts JGJV, Barbieri F, et al. Clinicopathologic Correlates of First-Line Pembrolizumab Effectiveness in Patients with Advanced NSCLC and a PD-L1 Expression of ≥ 50. Cancer Immunology, Immunotherapy. 2020;69(11):2209-21.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

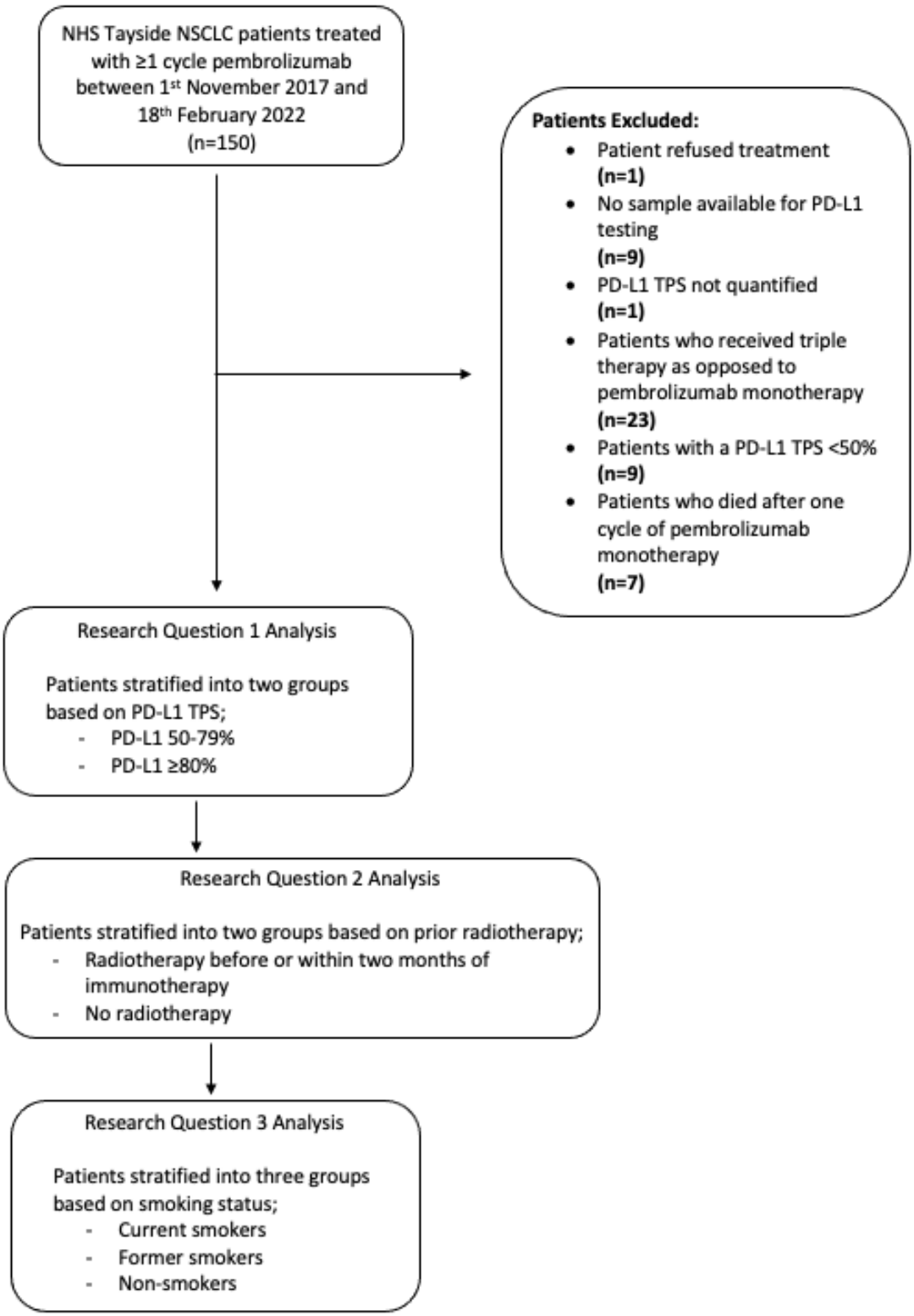
Footnotes

- **Contributors** ESM: conceptualisation, methodology, investigation, writing (original draft), visualisation; HAN: investigation, writing (original draft), visualisation; CJM and HKL: investigation, writing (review and editing); MJF and GS: conceptualisation, methodology, investigation, writing (original draft), visualisation, supervision. As guarantor, GS accepts full responsibility for the work, had access to the data and controlled the decision to publish.
- **Funding** The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors. This research was performed as part of an intercalated University of Dundee degree in Genetics, Cancer & Personalised Medicine.
- **Presentation** Some of this research has been previously presented at the CRUK Lung Cancer Conference 2022, Manchester, UK.
- **Competing interests** None declared.
- **Patient and public involvement** Patients and/or the public were not involved in the design, conduct, reporting or dissemination plans of this research.
- **Provenance and peer review** Not commissioned; externally peer reviewed.
- **Supplemental material** This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

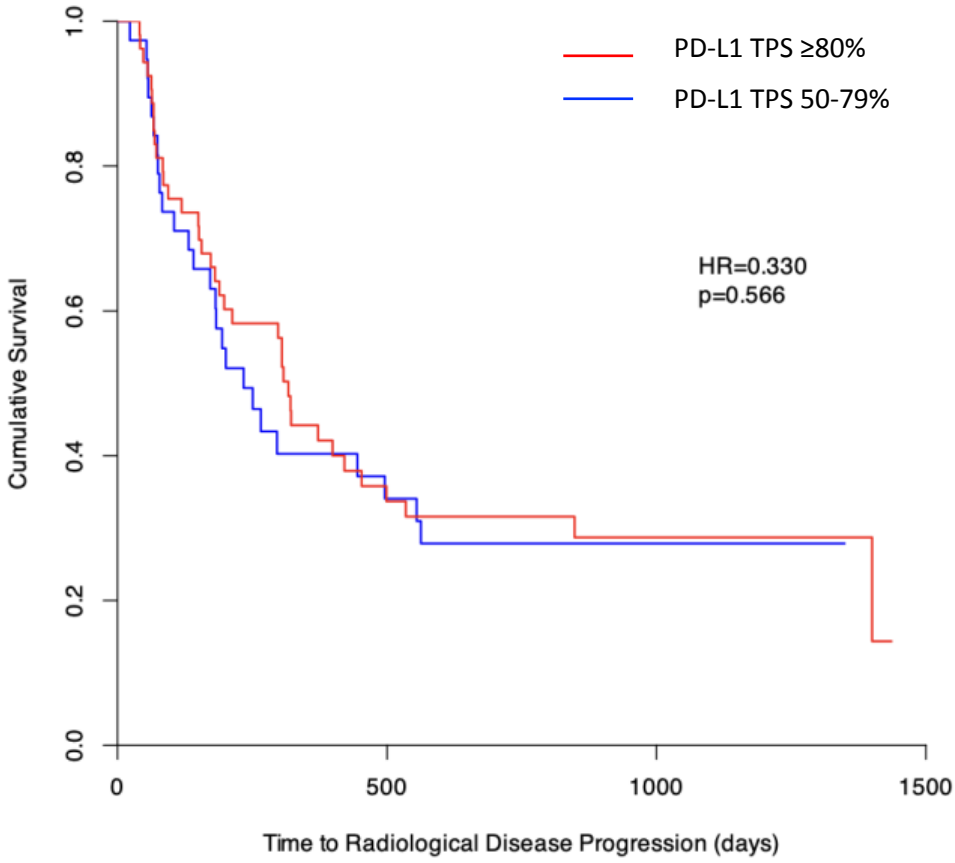
For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Mander *et al*, Figure 1

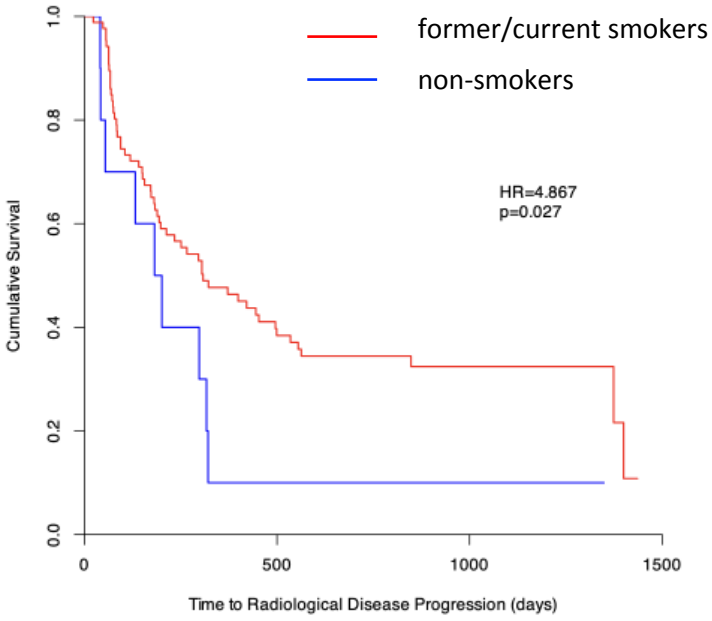


Mander *et al*, Figure 2

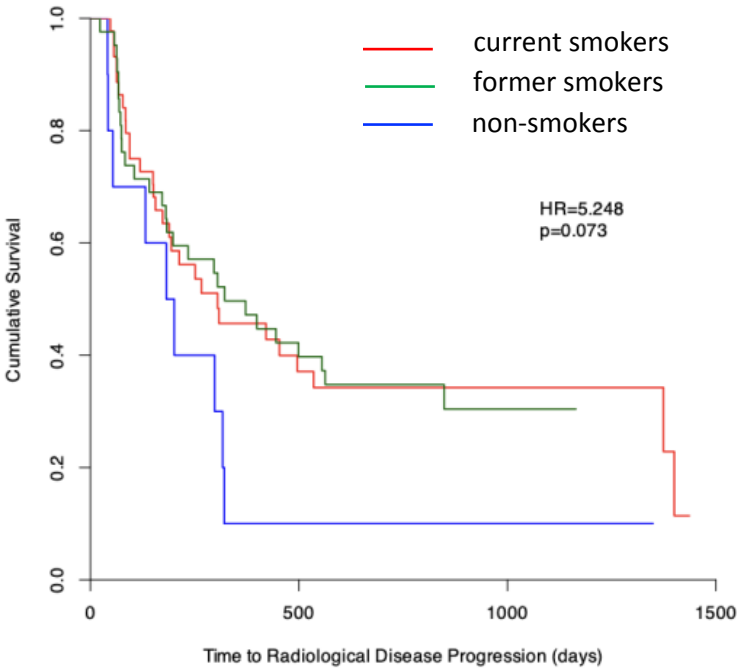


Mander *et al*, Figure 3

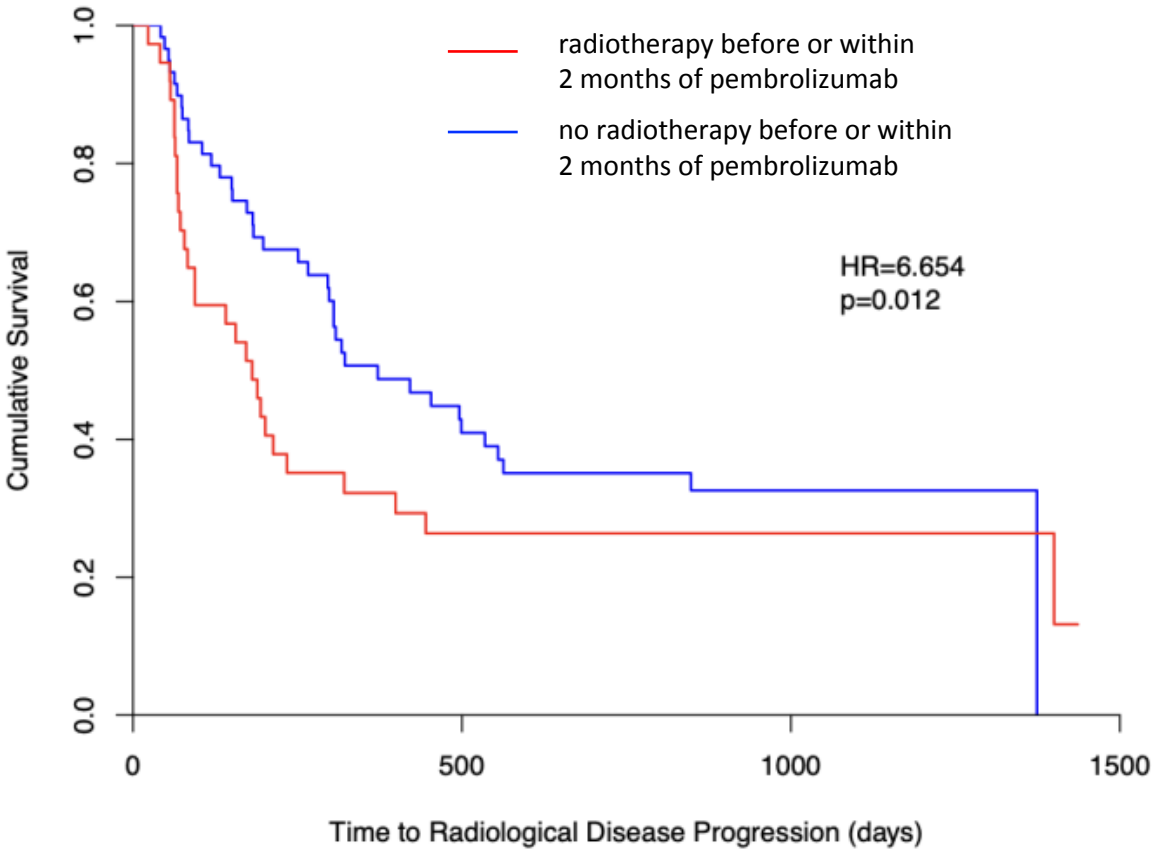
(A)



(B)



Mander *et al*, Figure 4



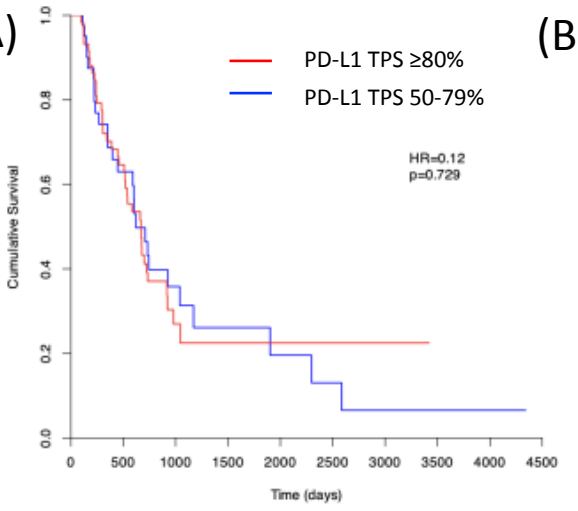
Mander *et al*, Supplemental Material, Table 1

Characteristics	PD-L1 TPS 50-79% (N = 40)	PD-L1 TPS ≥80% (N = 60)
Age - yr (at diagnosis)		
Median	67	68
Range	47-81	40-91
Sex		
Male - no. (%)	18 (45.0%)	26 (43.3%)
Female - no. (%)	22 (55.0%)	34 (56.7%)
Performance status		
0 - no. (%)	7 (17.5%)	8 (13.3%)
0-1 - no. (%)	0 (0%)	3 (5%)
1 - no. (%)	20 (50%)	35 (58.3%)
1-2 - no. (%)	0 (0%)	3 (5%)
2 - no. (%)	8 (20%)	10 (16.7%)
2-3 - no. (%)	3 (7.5%)	0 (0%)
3 - no. (%)	2 (5.0%)	1 (1.7%)
Smoking status		
Current - no. (%)	22 (55.0%)	23 (38.3%)
Former - no. (%)	12 (30.0%)	31 (51.7%)
Never - no. (%)	6 (15.0%)	6 (10.0%)
Histology		
Squamous cell carcinoma - no. (%)	12 (30.0%)	13 (21.7%)
Adenocarcinoma- no. (%)	25 (62.5%)	41 (68.3%)
Adenosquamous - no. (%)	1 (2.5%)	1 (1.7%)
Not specified - no. (%)	2 (5.0%)	5 (8.3%)
EGFR mutation status		
Positive - no. (%)	1 (2.5%)	1 (1.7%)
Negative - no. (%)	25 (62.5%)	43 (71.7%)
Unknown - no. (%)	14 (35.0%)	16 (26.7%)
ALK mutation status		
Positive - no. (%)	0 (0%)	0 (0%)
Negative - no. (%)	25 (62.5%)	43 (71.7%)
Unknown - no. (%)	15 (37.5%)	17 (28.3%)
ROS-1 mutation status		
Positive - no. (%)	0 (0%)	0 (0%)
Negative - no. (%)	10 (25.0%)	19 (31.7%)
Unknown - no. (%)	30 (75.0%)	41 (68.3%)
Stage		
I - no. (%)	1 (2.5%)	1 (1.7%)
II - no. (%)	3 (7.5%)	2 (3.3%)
III - no. (%)	10 (25.0%)	14 (23.3.%)
IV - no. (%)	26 (65.0%)	43 (71.7%)
Radiotherapy before or within two months of immunotherapy		
Radical - no. (%)	4 (10.0%)	6 (10.0%)
Palliative - no. (%)	12 (30.0%)	17 (28.3%)
Thoracic - no. (%)	4 (10.0%)	10 (16.7%)
Extra-thoracic - no. (%)	8 (20.0%)	7 (11.7%)

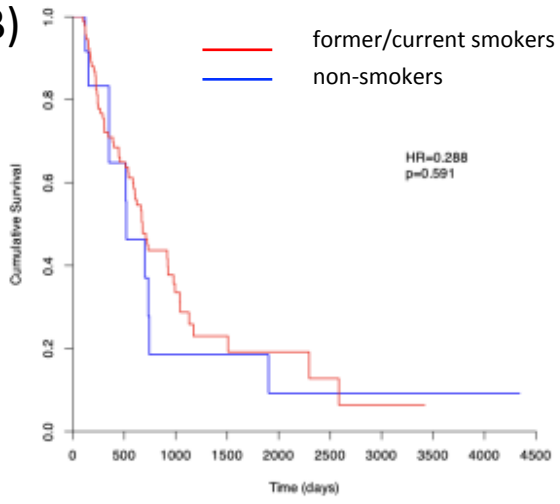
For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Mander *et al*, Supplemental Material, Appendix 1

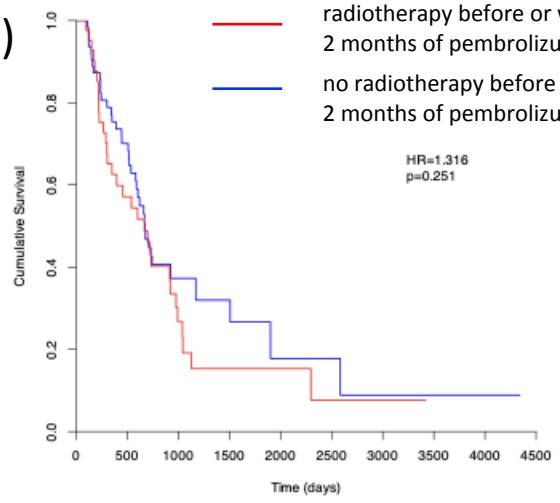
(A)



(B)



(C)



BMJ Open

Pembrolizumab Monotherapy for Non-Small Cell Lung Cancer (NSCLC): Can Patient Stratification be Improved in the Tayside Population? A Retrospective Cohort Study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2023-076715.R1
Article Type:	Original research
Date Submitted by the Author:	20-Sep-2023
Complete List of Authors:	Mander, Emily ; University of Dundee, School of Medicine Merrick, Christopher ; NHS Tayside, Tayside Cancer Centre Nicholson, Hugh ; University of Dundee, School of Medicine Lord, Hannah ; NHS Tayside, Tayside Cancer Centre Ferguson, Michelle ; NHS Tayside, Tayside Cancer Centre Smith, Gillian; University of Dundee, School of Medicine
Primary Subject Heading:	Oncology
Secondary Subject Heading:	Patient-centred medicine
Keywords:	RADIOTHERAPY, CHEMOTHERAPY, Clinical Decision-Making, ONCOLOGY, Respiratory tract tumours < ONCOLOGY

SCHOLARONE™
Manuscripts

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

For peer review only

Manuscript for submission to BMJ Open, June 2023_Revised September 2023

Pembrolizumab Monotherapy for Non-Small Cell Lung Cancer (NSCLC): Can Patient Stratification be Improved in the UK Tayside Population? A Retrospective Cohort Study

Emily S Mander[1], Christopher B Merrick[2], Hugh A Nicholson[3], Hannah K Lord[2], Michelle J Ferguson[2], Gillian Smith[3]

[1]School of Medicine, University of Dundee; [2]Tayside Cancer Centre; [3]Division of Cellular & Systems Medicine, School of Medicine, University of Dundee, Ninewells Hospital & Medical School, Dundee DD1 9SY

Correspondence to: Dr Gillian Smith, Division of Cellular & Systems Medicine, School of Medicine, University of Dundee, Jacqui Wood Cancer Centre, Ninewells Hospital & Medical School, James Arrott Drive, Dundee DD1 8SY. Email: g.smith@dundee.ac.uk, ORCID 0000-0001-9288-7566.

For peer review only

Abstract

Objective: Pembrolizumab is a PD-1 inhibitor used to treat advanced NSCLC patients with PD-L1 tumour proportion score (TPS) ≥50%. Further TPS-based stratification has not been evaluated in the UK, although smoking-induced tumour mutational burden and the immunogenic effects of prior radiotherapy are suggested to improve response.

Aims: To investigate if PD-L1 TPS ≥80%, smoking status or radiotherapy before or within 2 months of treatment influenced progression-free survival (PFS) in NSCLC patients treated with pembrolizumab monotherapy.

Methods: PD-L1 TPS, smoking status and radiotherapy exposure were compared in NSCLC patients in NHS Tayside (n=100) treated with pembrolizumab monotherapy between 1st November 2017 and 18th February 2022. Survival estimates were compared using log rank analysis, and Cox proportional hazards analysis used to investigate the influence of potential confounding factors, including tumour stage and performance status.

Results: PFS was not significantly different (log rank hazard ratio (HR)=0.330, p=0.566) comparing patients with PD-L1 TPS 50-79% and PD-L1 TPS ≥80%. Smokers had significantly improved PFS (log rank HR=4.867, p=0.027), while patients receiving radiotherapy had significantly decreased PFS (log rank HR=6.649, p=0.012). A Cox regression model confirmed that both radiotherapy (p=0.022) and performance status (p=0.009) were independent negative predictors of PFS.

Conclusions: More rigorous PD-L1 TPS stratification did not influence survival outcomes. Smoking history improved PFS, although was not an independent response predictor, while radiotherapy and performance status independently influenced clinical response. We suggest that further stratification of PD-L1 TPS is not warranted, while performance status and radiotherapy treatment may be additional clinically useful biomarkers of response to pembrolizumab in NSCLC patients.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Strengths and Limitations of this Study

- Following Caldicott Guardian approval, 150 NSCLC patients were identified in a single centre in NHS Tayside, UK, following a diagnosis of non-small cell lung cancer and treatment with at least one cycle of pembrolizumab therapy, between November 2017 and February 18th, 2022.

- Patients (n=50) were excluded from the study if tumour PD-L1 TPS was unknown or <50%, they refused treatment, died after one cycle of pembrolizumab therapy, or pembrolizumab was prescribed in combination with chemotherapy.

- PD-L1 TPS for each tumour, assessed by immunohistochemistry, radiotherapy prescribing information and self-reported smoking data (never/current/former smokers) was obtained from clinical records.

- The influence of PD-L1 TPS (comparing TPS 50-79% and TPS TPS≥80%) , radiotherapy and smoking status on PFS was assessed using Log-Rank analysis, and Cox proportional hazards models constructed to investigate whether significant conclusions were influenced by potential confounding variables, including performance status, stage and histology.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Introduction

Lung cancer, the third most common cancer in the UK and the principal cause of cancer mortality in both the UK and the USA (1) (2), is often diagnosed at late stage. Non-small cell lung cancer (NSCLC) is most commonly diagnosed, with a variety of histological types; adenocarcinoma (40%), squamous cell carcinoma (25%) and large cell carcinoma (10%) (3) (4). Advanced NSCLC (TNM stage III and IV) is treated with systemic anticancer therapy (SACT), as surgery is no longer possible (5). Chemotherapy offers poor survival outcomes in patients with advanced NSCLC, with a 1-year survival rate of around 30% (6). While subsets of NSCLCs have actionable targets including epidermal growth factor receptor (EGFR) mutations, anaplastic lymphoma kinase (ALK) translocations, and c-ROS oncogene 1 (ROS-1) rearrangements, the majority of NSCLCs do not express these oncogenic drivers (7).

Immune checkpoint inhibitors (ICI) targeting the programmed cell death protein-1/programmed cell death ligand-1 (PD-1/PD-L1) axis have revolutionised the treatment of advanced NSCLC, as they provide a stratified treatment option for patients with PD-L1 positive tumours but no other targetable mutations. PD-L1 expression is increased in NSCLC through aberrant signalling mechanisms resulting in T-cell inhibition which allows tumour cells to evade immune destruction (8) (9) (10).

Pembrolizumab is a monoclonal antibody which targets PD-1 on T-cells to disrupt the PD-1/PD-L1 axis (11) (12). Prescription of pembrolizumab in NSCLC is based on immunohistochemical assessment of % PD-L1 tumour proportion score (TPS) as a biomarker to stratify patients (13). In Scotland, Scottish Medicines Consortium (SMC) guidelines approve the use of pembrolizumab as first line monotherapy for advanced NSCLC in patients with PD-L1 TPS ≥50% with no EGFR mutations or ALK translocations. It is also licensed as second line monotherapy for patients with PD-L1 TPS ≥1% who have received at least one prior chemotherapy regime, and as first line treatment in combination with pemetrexed and platinum chemotherapy for advanced NSCLC patients with PD-L1 TPS <50%. Patients must not be eligible for alternative EGFR, ALK or ROS-1 targeted treatments as these can be targeted with specific inhibitors, such as the EGFR inhibitor gefitinib (14). The Keynote-010 clinical trial investigated superiority of pembrolizumab over docetaxel (OS HR 0·54, 95% CI 0·38–0·77, p=0.002, PFS HR 0·50, 95% CI 0·36–0·70, p=0.0001) (15) and confirmed improved response to pembrolizumab in patients with PD-L1 TPS ≥50%, while the Keynote-042 trial similarly reported improved pembrolizumab outcomes compared with investigator choice chemotherapy, when patients were stratified by TPS ≥50% (OS HR 0·69 (95% CI 0·56–0·85), p=0·0003, PFS HR 0·81 (95% CI 0·67–0·99), p=0·0170) (16).

While pembrolizumab monotherapy is a more effective treatment than chemotherapy for many NSCLC patients, it is associated with significant immune-related adverse effects, including thyroiditis, pneumonitis, colitis, nephritis, hypophysitis, hepatitis, encephalitis, myocarditis and severe cutaneous adverse reactions (SCARs) that can be severe and occasionally life-limiting (15) (17) (18). It is therefore important that the most appropriate patients are selected for pembrolizumab treatment. Disease response to pembrolizumab is routinely evaluated after two or three cycles of therapy and then every six to nine weeks thereafter. Response is evaluated radiologically, usually using CT scans, which are reported

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

using Response Evaluation Criteria in Solid Tumours (RECIST) criteria (7). Pembrolizumab therapy is associated with a rare treatment response known as pseudoprogression, where an initial increase in tumour burden is seen on imaging, with a subsequent reduction resulting in an overall decrease in tumour burden (19). The reported incidence of pseudoprogression in NSCLC patients treated with ICI is only 5% (20), although it is a significant clinical challenge as it is difficult to differentiate from true progression (20).

High mutational burden and associated molecular smoking signatures have been associated with increased efficacy of pembrolizumab therapy (21). Several studies have also linked cigarette smoking to high tumour PD-L1 expression (22) (23) (24) (25). For example, a prospective study in Canada involving 268 advanced NSCLC patients demonstrated that patients with PD-L1 TPS ≥50% who were smokers had a better response to anti-PD-1 immunotherapy than non-smokers. Objective response rate for current smokers was 36% compared to 26% in former smokers and 14% in non-smokers (p=0.02). Overall survival was also significantly increased in smokers compared to non-smokers. At 1-year post-diagnosis, 85.2% of current smokers were alive compared to 56.1% of former smokers and 42.6% of non-smokers (p=0.003) (26).

Radiotherapy can be used to treat NSCLC both palliatively and radically and has been hypothesised to have an immunostimulatory effect (27) (28), resulting from the release of damage-associated molecular pattern molecules (DAMPs) following tumour cell destruction by radiation. DAMPs activate dendritic cells which trigger the immune system to mount a specific T-cell response (29) (30), resulting in an “abscopal effect”, where tumour sites distant from the location of radiotherapy start to regress (31).

A secondary analysis of the Keynote-001 clinical trial of pembrolizumab in NSCLC investigated the effects of radiotherapy prior to pembrolizumab monotherapy and found that patients who had received prior radiotherapy had a significantly increased median progression-free (4.4 months compared to 2.1 months in the group who did not receive prior radiotherapy (p=0.019)) and overall survival (10.7 months compared to 5.3 months in patients who did not receive prior radiotherapy (p=0.026)). At 6 months progression-free survival was 49% in the prior radiotherapy group compared to 23% in patients that did not receive prior radiotherapy (p=0.019) (32).

The PEMBRO-RT Phase II clinical trial was designed to investigate whether stereotactic ablative radiotherapy (SABR) prior to pembrolizumab therapy resulted in enhanced treatment response in metastatic NSCLC, regardless of PD-L1 expression. 76 patients were randomised in a 1:1 ratio to receive either pembrolizumab monotherapy (control group) or SABR prior to pembrolizumab (experimental group). Median progression-free survival was 6.6 months in the SABR group compared to only 1.9 months in the control group, although this difference was not statistically significant (p=0.19). Similarly, median overall survival was 15.9 months in the SABR group compared to 7.6 months in the no radiotherapy group (p=0.16) (33).

As well as PD-L1 TPS, smoking and radiotherapy there are other important modifiers of outcome to consider for all cancer patients, including performance status and the stage and histology. Performance status is a measure of the functional status of a patient and is assessed

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

using the Eastern Cooperative Oncology Group Score (ECOG) Performance Status Scale, with scores from zero to five, where zero indicates no functional deficit and 5 confirms that the patient is deceased (34). Several studies have suggested that patients with performance status ≥2 have worse survival outcomes following pembrolizumab treatment than patients with performance status 0-1 (35) (36) (37).

This study aimed to investigate whether pembrolizumab patient selection could be refined by further sub-division of PD-L1 expression thresholds, and whether previous data describing a positive association of smoking on progression-free survival in NSCLC patients on pembrolizumab therapy was seen in the UK Tayside population. Based on current literature reporting potential immunostimulatory effects of radiotherapy, we also aimed to investigate the influence of radiotherapy on progression-free survival in NSLCC patients prescribed pembrolizumab in routine clinical practice, outwith a controlled clinical trial setting.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Methods

1. Study Approval

Caldicott Guardian Approval was received to allow collection of confidential NSCLC patient information in NHS Tayside.

2. Patient Selection

Study data was collected from NHS computers in Ward 32 Oncology, Ninewells Hospital & Medical School, Dundee between 31st January 2022 and 18th February 2022, with further follow up data collection from 5th January 2023 to 19th February 2023. All patient data was anonymised before inclusion in the study. 150 NSCLC patients were identified from the NHS Tayside oncology database following a diagnosis of non-small cell lung cancer and treatment with at least one cycle of pembrolizumab therapy between November 2017 and February 18th, 2022. Patients were excluded from the study if tumour PD-L1 TPS was unknown or <50%, they refused treatment, died after one cycle of pembrolizumab therapy, or pembrolizumab was prescribed in combination with chemotherapy (triple therapy). Demographic information for all patients, including age, sex, performance status, tumour histology, tumour stage and EGFR, ALK and ROS-1 status was obtained from the Chemocare database, ICE and Clinical Portal.

3. PD-L1 Expression Data

PD-L1 TPS for each tumour, assessed by immunohistochemistry, was obtained from pathology reports or reports from Tayside Lung Cancer Multi-disciplinary Team Meetings (MDTs), obtained from the ICE database. Patients were then stratified into two groups: PD-L1 TPS 50-79% and PD-L1 TPS ≥80%.

4. Radiotherapy Data

Oncology records, accessed through the Clinical Portal database, were used to document the date, type and location of any radiotherapy given. Patients were initially stratified into two groups: those who received radiotherapy at any time before or within two months of immunotherapy, and those who did not receive radiotherapy before or within two months of immunotherapy. Patients were then further sub-divided by palliative or radical radiotherapy, with patients receiving palliative radiotherapy further divided into two subgroups based on radiotherapy location (thoracic or extra thoracic).

5. Smoking Data

Self-reported smoking status was obtained from medical records using the Clinical Portal database. Patients were first divided into two groups: patients who had ever smoked and patients who had never smoked. Patients who had smoked were then further divided into current smokers and former smokers.

6. Study Outcomes

Due to the retrospective nature of the study, many patients went on to receive other forms of systemic anticancer therapy (SACT), so there were many potential confounding variables that could influence overall survival. Therefore, consistent with other similar retrospective cohort studies involving immunotherapy in NSCLC, progression-free survival (PFS) was used

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

as the primary outcome of the study. PFS was calculated as the time in days from the start of cycle one of pembrolizumab therapy to the date of radiological disease progression. Treatment response CT scans were carried out every six to nine weeks in this patient cohort. Overall survival, assessed as a secondary endpoint, was calculated as the time in days between the date of diagnosis and the date of death or census end point (February 18th, 2022).

7. Statistical Analysis

Statistical analysis was carried out using version 27 of the SPSS statistics programme (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0, Armonk, NY: IBM Corp). Baseline patient demographics were compared in patients with PD-L1 50-79% and PD-L1≥80% using Mann-Whitney tests for non-parametric data. Progression-free and overall survival were assessed using Log-Rank analysis, with Kaplan-Meier Survival Plots created using the ggplot2 and survival packages and Cairo function in the open-source R programming environment Version 2023.03.1+446 (38). If the Kaplan-Meier Plots produced significant results, further Cox proportional hazards models were constructed in SPSS to investigate whether significant conclusions were influenced by potential confounding variables, including performance status, stage and histology.

8. Patient and Public Involvement Statement

Patients or the public were not involved in the design, conduct, reporting or dissemination plans of our research.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Results

1. Patient Demographics

150 patients were initially assessed for inclusion in the study, however final analysis was carried out on 100 patients as 50 patients did not meet the inclusion criteria - 1 patient refused treatment, 9 patients did not have a sample available for PD-L1 testing, PD-L1 TPS was not quantified in 1 patient, 23 patients received triple therapy, 9 patients had PD-L1 TPS <50% and 7 patients died after one cycle of pembrolizumab (Figure 1). Patient demographics are further summarised in Supplemental Material, Table 1.

2. Does PD-L1 TPS 50-79% in comparison to ≥80% influence PFS or OS?

To investigate if stratification of NSCLC patients for pembrolizumab treatment could be further refined by very high PD-L1 TPS (≥80%), patients were separated into two groups; PD-L1 TPS 50-79% and PD-L1 TPS ≥80%, with PD-L1 TPS assessed as described in Methods. There was no significant difference comparing progression-free survival in NSCLC patients with PD-L1 TPS 50-79% and those with PD-L1 TPS ≥80% (HR=0.330, p=0.566) (Figure 2). Similarly, there was no significant difference in overall survival comparing patients with PD-L1 TPS 50-79% and those with PD-L1 TPS ≥80% (HR=0.120, p=0.729) (Supplemental Material, Figure 1A). In additional exploratory analysis, we increased the PD-L1 TPS threshold to 90%, comparing patients with PD-L1 TPS 50-89% and PD-L1 TPS ≥90%, but again found no significant differences in PFS or OS (data not shown).

3. Does smoking history influence survival outcomes in NSCLC patients prescribed pembrolizumab?

To investigate if smoking status had a significant impact on PFS, patients were sub-divided according to smoking status, as described in Methods. Patients who were smokers (defined as current or former smokers) had significantly longer progression-free survival compared to patients who were non-smokers (HR=4.867, p=0.027) (Figure 3A). Patients were then further subdivided into current smokers, former smokers and non-smokers, with no significant differences in PFS in current smokers and former smokers (HR=5.248, p=0.073) (Figure 3B). In contrast, no significant difference in overall survival was seen in patients who were smokers and those who were non-smokers (HR=0.288, p=0.591) (Supplemental Material, Figure 1B).

4. Does prior radiotherapy treatment influence survival outcomes in NSCLC patients prescribed pembrolizumab?

To investigate the influence of radiotherapy on progression-free survival, patients were categorised based on whether or not they had received radiotherapy before or within two months of pembrolizumab monotherapy, as described in Methods. In contrast to published data, patients who received radiotherapy had significantly decreased progression-free survival compared to patients who did not receive radiotherapy (HR=6.254, p=0.012) (Figure 4). Similar to our smoking data, there was no significant difference in overall survival between patients who received radiotherapy before or within two months of pembrolizumab monotherapy and those who did not (HR=1.316, p=0.251) (Supplemental Material, Figure 1C).

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

A Cox Regression Model was then used to investigate whether the significant smoking and radiotherapy associations reported above were modified by potential confounding factors including performance status, tumour stage and histology. Cox regression analysis confirmed that radiotherapy at any point before or within two months of pembrolizumab monotherapy (p=0.022) and performance status (0.009), but not stage (p=0.126), histology (p=0.827), PD-L1 TPS (p=0.568) or smoking status (p=0.081) were independent predictors of PFS in NSCLC patients treated with pembrolizumab (Supplementary Information, Appendix 2).

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Discussion

Approval of pembrolizumab has revolutionised the treatment of advanced and metastatic NSCLC, although treatment is expensive and patient selection limited to immunohistochemical assessment of tumour proportion score (TPS), with patients with PD-L1 TPS ≥50% currently eligible for treatment. To investigate whether more rigorous TPS stratification might influence treatment response in routine clinical practice, we compared PD-L1 TPS 50-79% and PD-L1 TPS ≥80% in a cohort of unselected NSCLC patients treated in a single centre, and further investigated whether clinical outcomes were influenced by smoking, previous radiotherapy exposure or could simply be predicted by performance status.

We first investigated whether further stratification of PD-L1 TPS might lead to improved clinical outcomes in NSCLC patients. For consistency with previous reports, we used PFS as our primary and OS as secondary analysis endpoint in order to limit additional sources of variation, as many patients received additional SACT following disease progression on pembrolizumab monotherapy. We found no significant difference in either progression-free (HR=0.330, p=0.566) or overall (HR=0.120, p=0.729) survival, comparing patients with PD-L1 TPS 50-79% and PD-L1 TPS ≥80% and in further analysis increasing the PD-L1 TPS threshold to 90% , suggesting that further TPS-based patient stratification may not be warranted. We chose to initially exclude 7 patients from our analysis as they died following 1 cycle of pembrolizumab, when it had not been possible to investigate disease progression by CT scan – to ensure that exclusion of these patients had not inadvertently influenced our survival analysis, we confirmed that our OS data was similar in the extended dataset. Our data contrasts with the results of an American retrospective study (n=187 patients), which reported an association of PD-L1 TPS ≥90% with significantly improved PFS (14.5 months vs 4.1 months, HR=0.50, p<0.01) (39). However, similar to our own data, a retrospective cohort study in Japan (n=149 patients), comparing PFS in patients with PD-L1 TPS 50-89% and 90-100% reported no significant difference in progression-free survival (HR=0.78, p=0.34). PFS in the Japanese study at 120 days was 64.4% in PD-L1 TPS 50-89% patients and 63.0% in PD-L1 TPS 90-100% patients (HR=1.03, p=0.09) (40), similar to our own data which reports PFS of 70% at 120 days in the PD-L1 50-79% group and 76% in the PD-L1 ≥80% group (p=0.566). Both the American and Japanese studies used higher (≥90%) PD-L1 TPS to stratify patients, and it is important to note that the American study reported TPS using four different antibodies due to differences in practice between institutions. This observation highlights the limitations of PD-L1 as a quantitative biomarker. Although testing is standardised across Scotland, using the same Dako 22C3 antibody reported in the early Keynote trials (41) (17), PD-L1 TPS is routinely reported following expert pathologist assessment of immunohistochemical staining, with associated inherent variation between centres and reporting pathologists (42). Tumour heterogeneity at diagnosis is additionally recognised to significantly influence PD-L1 expression (43), and it is likely that expression varies further during disease progression and treatment. Despite these limitations, baseline PD-L1 TPS assessed from the initial diagnostic biopsy is currently routinely used to inform patient selection for immunotherapy. We highlight the need in future studies to develop more quantitative methods for PD-L1 assessment, to facilitate more rigorous evaluation of the potential of TPS as a predictive and prognostic biomarker.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Our data initially confirmed previous reports (23) (24) (44), suggesting that patients who were current or former smokers had significantly longer PFS than non-smokers (HR=4.867, p=0.027). Importantly, PFS in current smokers and former smokers was not significantly different (HR=5.248, p=0.073), suggesting that any smoking history has the potential to modify pembrolizumab response. Consistent with our data, a recent meta-analysis investigating the impact of smoking status on targeted therapy in NSCLC in Phase III clinical trials reported that smokers had significantly extended PFS following immune checkpoint inhibitor treatment (HR=1.81, p=0.004) (44), with additional meta-analyses reporting similar conclusions (23) (24). It is also important to note, however, that our extended Cox regression analysis did not confirm smoking history as an independent predictor of pembrolizumab response in NSCLC, and that the influence of confounding factors has not always been previously reported. Although it is logical that smoking may increase tumour mutation burden (TMB) and, as a consequence, increase immunogenicity and improve response to immunotherapy, it is important to acknowledge that TMB has not been routinely assessed in significant numbers of patients outwith the clinical trial setting, and that results from some previous studies do not support this hypothesis (21). The use of smoking status as a biomarker for pembrolizumab response additionally raises important ethical issues as smoking cessation is an important part of the clinical management of lung cancer, as it improves outcomes and reduces the risk of the development of further cancers (45) (46) and other diseases associated with smoking such as cardiovascular disease and chronic obstructive pulmonary disease (COPD) (47). Further, in this and previous studies, patients were identified as smokers or non-smokers based on self-reported smoking history. Verification of smoking status, for example using biochemical confirmation of serum cotinine levels, is recommended but is challenging outwith the clinical trial setting (48), and self-reported smoking history is more likely to be under rather over-represented, in turn under-estimating pembrolizumab response predictions in smokers. Serum cotinine has been successfully used to confirm self-reported smoking status to identify eligible patients for lung cancer screening (48) and can also be used in patients using electronic cigarettes containing nicotine (49) (50). We highlight the need to include more quantitative and objective assessment of smoking history in future studies to investigate whether the modifying effect on ICI response in NSCLC patients is dose-dependent and whether smoking status and TPS are independent risk modifiers.

Our analysis suggests that NSCLC patients receiving radiotherapy before or within two months of pembrolizumab monotherapy had significantly decreased PFS compared to patients who did not receive radiotherapy (HR=6.254, p=0.012), in contrast to the findings of the Keynote-001 clinical trial (32) which reported that radiotherapy increased the efficacy of immunotherapy, possibly due to the abscopal effect (51). Further studies, however, including a retrospective multicentre study evaluating the effects of palliative radiotherapy before or within three months of anti-PD-1 therapy reported no significant difference in PFS, comparing patients who had received radiotherapy and those who had not (3.2 months vs 2.0 months, p=0.515) (52), while the PEMBRO-RT trial also reported no significant difference in PFS in patients who received SABR prior to pembrolizumab therapy and those who did not (1.9 months vs 6.6 months, p=0.19), although the data suggested that the possible benefit of prior radiotherapy should be further investigated in a larger dataset (53). We acknowledge that patients receiving radiotherapy within 2 months of pembrolizumab in our study may have had more advanced disease, or may have progressed more quickly, although tumour stage at diagnosis was not independently predictive of PFS.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

In contrast to previously reported clinical trial data, the majority of patients in the current study received palliative radiotherapy (usually 8Gy in 1 fraction or 20Gy in 5 fractions (54)) rather than SABR. It is therefore possible that palliative radiotherapy does not potentiate immunogenicity in NSCLC patients, as most previous literature reports on the influence of higher dose SABR on immunotherapy outcomes (55). As many of our study patients had symptomatic metastases, it is also possible that the modifying effect of radiotherapy we report, while independently predictive of survival outcomes, may simply represent a surrogate marker for performance status. Many NSCLC patients are additionally prescribed steroids, either to alleviate tumour compression or the side effects of immunotherapy. Steroid use is known to suppress the immune system and may therefore further modify responses to both radiotherapy and immunotherapy (56). We highlight the need to investigate the potential modifying effect of steroid prescription in future studies, and also the potential modifying effect of radiotherapy and pembrolizumab scheduling, as tumour repopulation post radiotherapy may further influence pembrolizumab response (57) (58). It is also important to ensure that CT scan reporting is standardised as far as is practicable in routine clinical practice. In the Keynote-024 clinical trial, for example, CT scans were all reported according to RECIST criteria, by a radiologist independent from the trial (8). While undoubtedly increasing the accuracy of clinical response estimates, greater variation in CT reporting in routine clinical practice is inevitable, even in a single centre. Radiological response assessment is particularly important following immunotherapy treatment due to pseudo-progression, where an initial apparent increase in tumour burden due to accumulation of immune cells causing an inflammatory response result in enlargement of neoplastic lesions (19), followed by subsequent regression (59), and is difficult to differentiate from true disease progression through initial imaging (20) (60). To address this relatively rare complication (incidence <6% in NSCLC patients), revised Response Evaluation Criteria in Solid Tumours (RECIST) guidelines, iRECIST, were developed in 2017 to improve reporting in immunotherapy clinical trials (61).

Importantly, despite these acknowledged sources of variation in biomarker and radiological assessment, our data highlights that performance status is an independent predictor of PFS (p=0.009). We assessed outcomes in all NSCLC patients treated with pembrolizumab (performance status 0-3), in contrast to more restricted clinical trials where, for example, only patients with performance status 0-1 were included in the Keynote-024 clinical trial (17), and the PePS2 single arm Phase 2 trial evaluated pembrolizumab response in patients with PS ≥2 (62). Consistent with our findings, several previous studies have reported that patients with PS ≥2 have reduced survival outcomes (35) (36) (37), while a recent Italian multicentre retrospective study confirmed that performance status was an independent predictor of poor clinical outcome (63).

In conclusion, therefore, our data confirms that more rigorous stratification of NSCLC patients by PD-L1 TPS did not influence survival outcomes. Smoking status (current or previous smoker) significantly improved PFS, although was not an independent predictor of survival. In contrast, radiotherapy treatment at any point before or within two months of pembrolizumab therapy independently adversely influenced PFS, and performance status was shown to be an independent predictor of clinical response. We suggest that further stratification of PD-L1 TPS may not be warranted, the modifying effects of radiotherapy

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

require further investigation in carefully controlled future studies, and performance status in addition to the currently used PD-L1 TPS ≥50% may be a clinically useful biomarker of response to pembrolizumab in NSCLC patients.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Figure Legends

Figure 1: Patient Selection and Demographics
150 NSCLC patients were initially identified in NHS Tayside, between 31st January 2022 and 18th February 2022. 50 patients were excluded from the study as they failed to meet the inclusion criteria, for the reasons indicated. Patients were classified as smokers if they were current or ex-smokers, based on self-reported smoking status.

Figure 2: Further patient stratification by PD-L1 TPS does not influence PFS
Log-Rank analysis, represented as Kaplan-Meier survival plots was used to compare PFS in NSCLC patients with PD-L1 TPS ≥80% (red) and PD-L1 TPS 50-79% (blue).

Figure 3: Smoking history influences PFS in NSCLC patients prescribed pembrolizumab
Log-Rank analysis, represented as Kaplan-Meier survival plots was used to compare PFS in (A) smokers (former and current; red) and non-smokers (blue) and in (B) current smokers (red), former smokers (green), and non-smokers (blue).

Figure 4: Prior Radiotherapy influences PFS in NSCLC patients prescribed pembrolizumab
Log-Rank analysis, represented as Kaplan-Meier survival plots was used to compare PFS in NSCLC patients who received radiotherapy before or within two months of pembrolizumab (red) and those who did not receive radiotherapy in that time frame (blue).

Supplemental Material:

Supplemental Material, Figure 1A: Further patient stratification by PD-L1 TPS does not influence OS in NSCLC patients
Log-Rank analysis, represented as Kaplan-Meier survival plots was used to compare OS in NSCLC patients with PD-L1 TPS ≥80% (red) and PD-L1 TPS 50-79% (blue).

Supplemental Material, Figure 1B: Smoking history did not influence OS in NSCLC patients prescribed pembrolizumab
Log-Rank analysis, represented as Kaplan-Meier survival plots was used to compare OS in (A) smokers (former and current; red) and non-smokers (blue).

Supplemental Material, Figure 1C: Prior Radiotherapy did not influence OS in NSCLC patients prescribed pembrolizumab
Log-Rank analysis, represented as Kaplan-Meier survival plots was used to compare OS in NSCLC patients who received radiotherapy before or within two months of pembrolizumab (red) and those who did not receive radiotherapy in that time frame (blue).

Table 1: Summary of Patient demographics

Table 2: Cox Regression analysis

For peer review only

References:

1. Cancer Research UK. Lung Cancer Statistics, 2018 [Available from: https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/lung-cancer.]
2. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. CA Cancer J Clin. 2022;72(1):7-33.
3. NHS. Lung Cancer, 2019 [Available from: https://www.nhs.uk/conditions/lung-cancer/.]
4. National Cancer Institue. Non-Small Cell Lung Cancer Treatment: National Cancer Institute; 2022 [Available from: https://www.cancer.gov/types/lung/hp/non-small-cell-lung-treatment-pdq#_359.]
5. Molina JR, Yang P, Cassivi SD, Schild SE, Adjei AA. Non-Small Cell Lung Cancer: Epidemiology, Risk Factors, Treatment, and Survivorship. Mayo Clin Proc. 2008;83(5):584-94.
6. Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, et al. Comparison of Four Chemotherapy Regimens for Advanced Non–Small-Cell Lung Cancer. New England Journal of Medicine. 2002;346(2):92-8.
7. Planchard D, Popat S, Kerr K, Novello S, Smit EF, Faivre-Finn C, et al. Metastatic Non-Small Cell Lung Cancer: ESMO Clinical Practice Guidelines for Diagnosis, Treatment and Follow-up. Annals of Oncology. 2018;29(Supplement_4):iv192-iv237.
8. Ghosh C, Luong G, Sun Y. A Snapshot of the PD-1/PD-L1 Pathway. Journal of Cancer. 2021;12(9):2735-46.
9. Jiang Y, Chen M, Nie H, Yuan Y. PD-1 and PD-L1 in Cancer Immunotherapy: Clinical Implications and Future Considerations. Human Vaccines & Immunotherapeutics. 2019;15(5):1111-22.
10. Hanahan D, Weinberg RA. Hallmarks of Cancer: The Next Generation. Cell. 2011;144(5):646-74.
11. Kwok G, Yau TC, Chiu JW, Tse E, Kwong YL. Pembrolizumab (Keytruda). Hum Vaccin Immunother. 2016;12(11):2777-89.
12. BNF. Pembrolizumab | Drug | BNF content published by NICE: NICE; [Available from: https://bnf.nice.org.uk/drug/pembrolizumab.html.]
13. De Marchi P, Leal LF, Duval da Silva V, da Silva ECA, Cordeiro de Lima VC, Reis RM. PD-L1 Expression by Tumor Proportion Score (TPS) and Combined Positive Score (CPS) are Similar in Non-Small Cell Lung Cancer (NSCLC). Journal of Clinical Pathology. 2021;74(11):735-40.
14. SMC. Pembrolizumab (Keytruda): Scottish Medicines Consortium; [Available from: https://www.scottishmedicines.org.uk/medicines-advice/pembrolizumab-keytruda-fullsubmission-123917/.]
15. Herbst RS, Baas P, Kim DW, Felip E, Pérez-Gracia JL, Han JY, et al. Pembrolizumab Versus Docetaxel for Previously Treated, PD-L1-Positive, Advanced Non-Small-Cell Lung Cancer (KEYNOTE-010): A Randomised Controlled trial. Lancet. 2016;387(10027):1540-50.
16. Gandhi L, Rodríguez-Abreu D, Gadgeel S, Esteban E, Felip E, De Angelis F, et al. Pembrolizumab Plus Chemotherapy in Metastatic Non-Small-Cell Lung Cancer. N Engl J Med. 2018;378(22):2078-92.
17. Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, et al. Pembrolizumab Versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. N Engl J Med. 2016;375(19):1823-33.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

18. EMA. Keytruda - Summary of Product Characteristics [Available from: https://www.ema.europa.eu/en/documents/product-information/keytruda-epar-product-information_en.pdf.]
19. Jia W, Gao Q, Han A, Zhu H, Yu J. The Potential Mechanism, Recognition and Clinical Significance of Tumor Pseudoprogression after Immunotherapy. Cancer Biol Med. 2019;16(4):655-70.
20. Park HJ, Kim KW, Pyo J, Suh CH, Yoon S, Hatabu H, et al. Incidence of Pseudoprogression During Immune Checkpoint Inhibitor Therapy for Solid Tumors: A Systematic Review and Meta-Analysis. Radiology. 2020;297(1):87-96.
21. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Mutational Landscape Determines Sensitivity to PD-1 Blockade in Non-Small Cell Lung Cancer. Science. 2015;348(6230):124-8.
22. Norum J, Nieder C. Tobacco Smoking and Cessation and PD-L1 Inhibitors in Non-Small Cell Lung Cancer (NSCLC): A Review of the Literature. ESMO Open. 2018;3(6):e000406.
23. Zhao W, Jiang W, Wang H, He J, Su C, Yu Q. Impact of Smoking History on Response to Immunotherapy in Non-Small-Cell Lung Cancer: A Systematic Review and Meta-Analysis. Front Oncol. 2021;11:703143.
24. Lee KWC, Lord SJ, Kasherman L, Marschner I, Stockler M, Gralla R, et al. The Impact of Smoking on the Effectiveness of Immune Checkpoint Inhibitors — A Systematic Review and Meta-analysis. Acta Oncologica. 2020;59(1):96-100.
25. Abdel-Rahman O. Smoking and EGFR Status May Predict Outcomes of Advanced NSCLC Treated with PD-(L)1 Inhibitors Beyond First Line: A Meta-analysis. The Clinical Respiratory Journal. 2018;12(5):1809-19.
26. Li JJN, Karim K, Sung M, Le LW, Lau SCM, Sacher A, et al. Tobacco Exposure and Immunotherapy Response in PD-L1 Positive Lung Cancer Patients. Lung Cancer. 2020;150:159-63.
27. SIGN. Management of Lung Cancer. 2014.
28. Reynders K, Illidge T, Siva S, Chang JY, De Ruysscher D. The Abscopal Effect of Local Radiotherapy: Using Immunotherapy to Make a Rare Event Clinically Relevant. Cancer Treatment Reviews. 2015;41(6):503-10.
29. Bhalla N, Brooker R, Brada M. Combining Immunotherapy and Radiotherapy in Lung Cancer. Journal of Thoracic Disease. 2018;10(S13):S1447-S60.
30. Corke L, Sacher A. New Strategies and Combinations to Improve Outcomes in Immunotherapy in Metastatic Non-Small-Cell Lung Cancer. Current Oncology. 2021;29(1):38-55.
31. Turgeon GA, Weickhardt A, Azad AA, Solomon B, Siva S. Radiotherapy and Immunotherapy: A Synergistic Effect in Cancer Care. Medical Journal of Australia. 2019;210(1):47-53.
32. Shaverdian N, Lisberg AE, Bornazyan K, Veruttipong D, Goldman JW, Formenti SC, et al. Previous Radiotherapy and the Clinical Activity and Toxicity of Pembrolizumab in the Treatment of Non-Small-Cell Lung Cancer: A Secondary Analysis of the KEYNOTE-001 Phase 1 Trial. The Lancet Oncology. 2017;18(7):895-903.
33. Welsh J, Menon H, Chen D, Verma V, Tang C, Altan M, et al. Pembrolizumab With or Without Radiation Therapy for Metastatic Non-Small Cell Lung Cancer: A Randomized Phase I/II Trial. Journal for ImmunoTherapy of Cancer. 2020;8(2):e001001.
34. ECOG-ACRIN Cancer Research Group. ECOG Peformance Status Scale [Available from: https://ecog-acrin.org/resources/ecog-performance-status/.]

For peer review only

35. Tamiya M, Tamiya A, Hosoya K, Taniguchi Y, Yokoyama T, Fukuda Y, et al. Efficacy and Safety of Pembrolizumab as First-Line Therapy in Advanced Non-Small Cell Lung Cancer With At Least 50% PD-L1 Positivity: A Multicenter Retrospective Cohort Study (HOPE-001). Investigational new drugs. 2019;37(6):1266-73.
36. Sehgal K, Gill RR, Widick P, Bindal P, McDonald DC, Shea M, et al. Association of Performance Status With Survival in Patients With Advanced Non-Small Cell Lung Cancer Treated With Pembrolizumab Monotherapy. JAMA Netw Open. 2021;4(2):e2037120.
37. Addeo A, Metro G, Signorelli D, Economopoulou P, Roila F, Banna GL, et al. Poor Performance Status and Front-Line Pembrolizumab in Advanced Non-Small-Cell Lung Cancer (NSCLC) Patients with PD-L1>50%. Journal of Clinical Oncology. 2020;38(15_suppl):e21651-e.
38. R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
39. Aguilar EJ, Ricciuti B, Gainor JF, Kehl KL, Kravets S, Dahlberg S, et al. Outcomes to First-Line Pembrolizumab in Patients with Non-Small-Cell Lung Cancer and Very High PD-L1 Expression. Ann Oncol. 2019;30(10):1653-9.
40. Edahiro R, Kanazu M, Kurebe H, Mori M, Fujimoto D, Taniguchi Y, et al. Clinical outcomes in Non-Small Cell Lung Cancer Patients with an Ultra-High Expression of Programmed Death Ligand-1 Treated Using Pembrolizumab As A First-Line Therapy: A Retrospective Multicenter Cohort Study In Japan. PloS one. 2019;14(7):e0220570-e.
41. Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, et al. Pembrolizumab for the Treatment of Non–Small-Cell Lung Cancer. New England Journal of Medicine. 2015;372(21):2018-28.
42. Ming Sound Tsao KMK, Sanja Dacic, Yasushi Yatabe, Fred R. Hirsch. IASLC Atlas of PD-L1 Immunohistochemistry Testing in Lung Cancer: International Association for the Study of Lung Cancer.
43. Munari E, Mariotti FR, Quatrini L, Bertoglio P, Tumino N, Vacca P, et al. PD-1/PD-L1 in Cancer: Pathophysiological, Diagnostic and Therapeutic Aspects. International Journal of Molecular Sciences. 2021;22(10):5123.
44. Li X, Huang C, Xie X, Wu Z, Tian X, Wu Y, et al. The Impact of Smoking Status on the Progression-Free Survival of Non-Small Cell Lung Cancer Patients Receiving Molecularly Target Therapy or Immunotherapy Versus Chemotherapy: A Meta-Analysis. Journal of Clinical Pharmacy and Therapeutics. 2021;46(2):256-66.
45. Luo SJ, Choi E, Aredo JV, Wilkens LR, Tammemägi MC, Le Marchand L, et al. Smoking Cessation After Lung Cancer Diagnosis and the Risk of Second Primary Lung Cancer: The Multiethnic Cohort Study. JNCI Cancer Spectr. 2021;5(5).
46. Caini S, Del Riccio M, Vettori V, Scotti V, Martinoli C, Raimondi S, et al. Quitting Smoking At or Around Diagnosis Improves the Overall Survival of Lung Cancer Patients: A Systematic Review and Meta-Analysis. J Thorac Oncol. 2022;17(5):623-36.
47. NHS. What Are The Health Risks Of Smoking? : NHS; 2018 [Available from: https://www.nhs.uk/common-health-questions/lifestyle/what-are-the-health-risks-of-smoking/.]
48. Liu B, Henschke CI, Flores RM, Taioli E. Serum Cotinine Verification of Self-Reported Smoking Status Among Adults Eligible for Lung Cancer Screening in the 1999-2018 National Health and Nutrition Examination Survey. Lung Cancer. 2020;144:49-56.
49. Vélez de Mendizábal N, Jones DR, Jahn A, Bies RR, Brown JW. Nicotine and Cotinine Exposure from Electronic Cigarettes: A Population Approach. Clin Pharmacokinet. 2015;54(6):615-26.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

50. Rapp JL, Alpert N, Flores RM, Taioli E. Serum Cotinine Levels and Nicotine Addiction Potential of E-Cigarettes: An NHANES Analysis. Carcinogenesis. 2020;41(10):1454-9.
51. Theelen WS, De Jong MC, Baas P. Synergizing Systemic Responses by Combining Immunotherapy with Radiotherapy in Metastatic Non-Small Cell Lung Cancer: The Potential of the Abscopal Effect. Lung Cancer. 2020;142:106-13.
52. Samuel E, Lie G, Balasubramanian A, Hiong A, So Y, Voskoboynik M, et al. Impact of Radiotherapy on the Efficacy and Toxicity of anti-PD-1 Inhibitors in Metastatic NSCLC. Clinical Lung Cancer. 2021;22(3):e425-e30.
53. Theelen WSME, Peulen HMU, Lalezari F, Van Der Noort V, De Vries JF, Aerts JGJV, et al. Effect of Pembrolizumab After Stereotactic Body Radiotherapy vs Pembrolizumab Alone on Tumor Response in Patients With Advanced Non–Small Cell Lung Cancer. JAMA Oncology. 2019;5(9):1276.
54. Lord H. Clinical Management Protocol - Non-Surgical Management of Non-Small Cell Lung Cancer: NHS Tayside; 2015 [Available from: https://www.nhstaysideadtc.scot.nhs.uk/tapg%20html/Specialist%20Lists/Oncology-Haematology/Protocols/Nonsmall%20cell%20lung%20prot.pdf.]
55. Damen PJJ, Verhoeff JJC. Efficacy Of Stereotactic Ablative Radiotherapy (SABR) During Anti-PD-1 In Oligoprogressive Non-Small Cell Lung Cancer And Melanoma-A Prospective Multicenter Observational Study Pointing Out New Unmet Needs. Transl Cancer Res. 2023;12(3):688-91.
56. Petrelli F, Signorelli D, Ghidini M, Ghidini A, Pizzutilo EG, Ruggieri L, et al. Association of Steroids Use With Survival In Patients Treated With Immune Checkpoint Inhibitors: A Systematic Review And Meta-Analysis. Cancers (Basel). 2020;12(3).
57. Ng WL, Huang Q, Liu X, Zimmerman M, Li F, Li CY. Molecular Mechanisms Involved In Tumor Repopulation After Radiotherapy. Transl Cancer Res. 2013;2(5):442-8.
58. Pajonk F, Vlashi E, McBride WH. Radiation Resistance Of Cancer Stem Cells: The 4 R's Of Radiobiology Revisited. Stem Cells. 2010;28(4):639-48.
59. Ma Y, Wang Q, Dong Q, Zhan L, Zhang J. How to Differentiate Pseudoprogression from True Progression in Cancer Patients Treated with Immunotherapy. Am J Cancer Res. 2019;9(8):1546-53.
60. Zhou L, Zhang M, Li R, Xue J, Lu Y. Pseudoprogression and Hyperprogression In Lung Cancer: A Comprehensive Review Of Literature. Journal of Cancer Research and Clinical Oncology. 2020;146(12):3269-79.
61. Seymour L, Bogaerts J, Perrone A, Ford R, Schwartz LH, Mandrekar S, et al. iRECIST: Guidelines For Response Criteria For Use in Trials Testing Immunotherapeutics. Lancet Oncol. 2017;18(3):e143-e52.
62. Middleton G, Brock K, Savage J, Mant R, Summers Y, Connibear J, et al. Pembrolizumab in Patients with Non-Small-Cell Lung Cancer of Performance Status 2 (PePS2): A Single Arm, Phase 2 Trial. The Lancet Respiratory Medicine. 2020;8(9):895-904.
63. Cortellini A, Tiseo M, Banna GL, Cappuzzo F, Aerts JGJV, Barbieri F, et al. Clinicopathologic Correlates of First-Line Pembrolizumab Effectiveness in Patients with Advanced NSCLC and a PD-L1 Expression of ≥ 50. Cancer Immunology, Immunotherapy. 2020;69(11):2209-21.

For peer review only

Footnotes

- **Contributors** ESM: conceptualisation, methodology, investigation, writing (original draft), visualisation; HAN: investigation, writing (original draft), visualisation; CJM and HKL: investigation, writing (review and editing); MJF and GS: conceptualisation, methodology, investigation, writing (original draft), visualisation, supervision. As guarantor, GS accepts full responsibility for the work, had access to the data and controlled the decision to publish.
- **Funding** The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors. This research was performed as part of an intercalated University of Dundee degree in Genetics, Cancer & Personalised Medicine.
- **Presentation** Some of this research has been previously presented at the CRUK Lung Cancer Conference 2022, Manchester, UK.
- **Competing interests** None declared.
- **Patient and public involvement** Patients and/or the public were not involved in the design, conduct, reporting or dissemination plans of this research.
- **Provenance and peer review** Not commissioned; externally peer reviewed.
- **Supplemental material** This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Mander *et al*, Figure 1

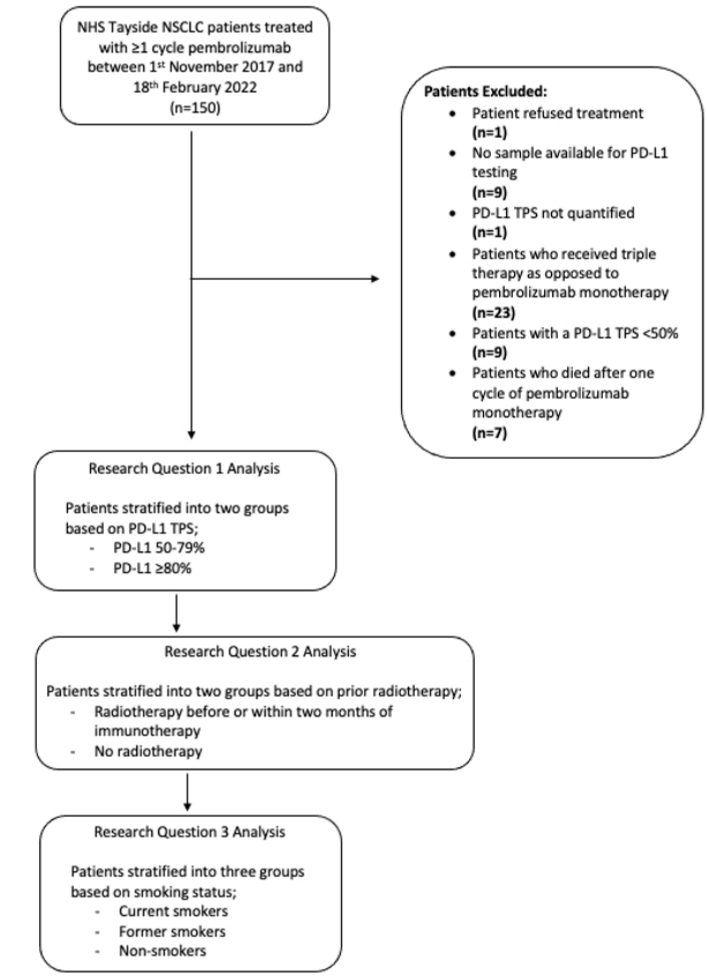


Figure 1: Patient Selection and Demographics

190x275mm (96 x 96 DPI)

Mander *et al*, Figure 2

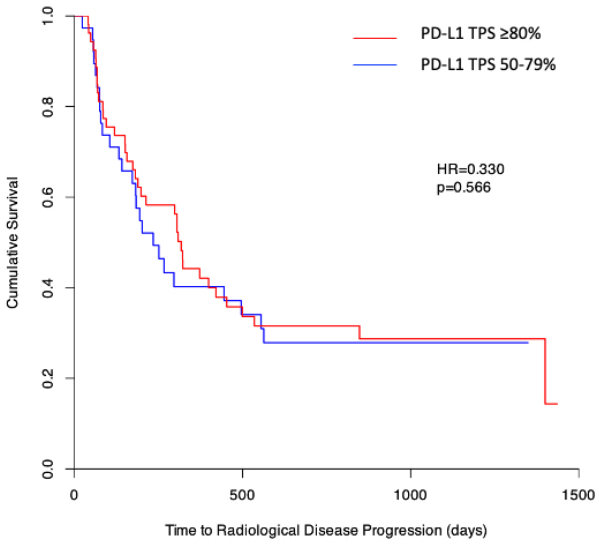


Figure 2: Further patient stratification by PD-L1 TPS does not influence PFS

190x275mm (96 x 96 DPI)

Mander *et al*, Figure 3

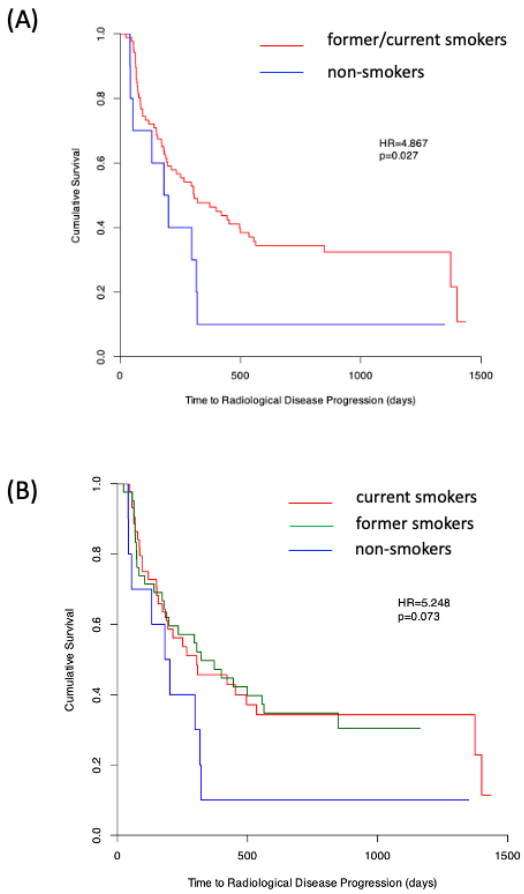


Figure 3: Smoking history influences PFS in NSCLC patients prescribed pembrolizumab

190x275mm (96 x 96 DPI)

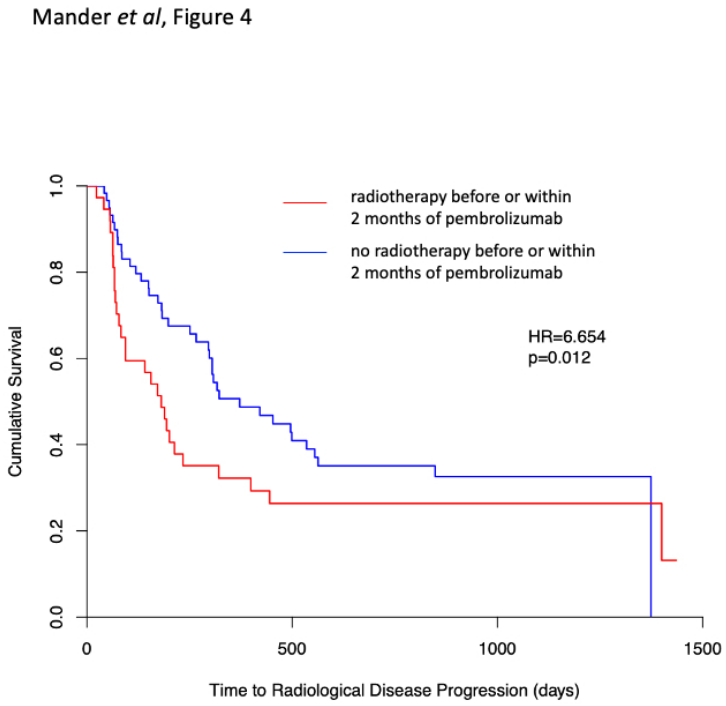


Figure 4: Prior Radiotherapy influences PFS in NSCLC patients prescribed pembrolizumab

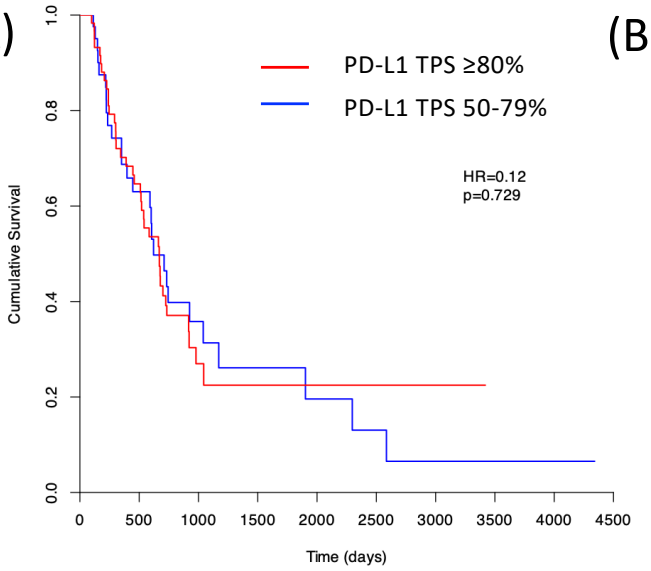
190x275mm (96 x 96 DPI)

Mander *et al*, Supplemental Material, Table 1
Summary of Patient demographics

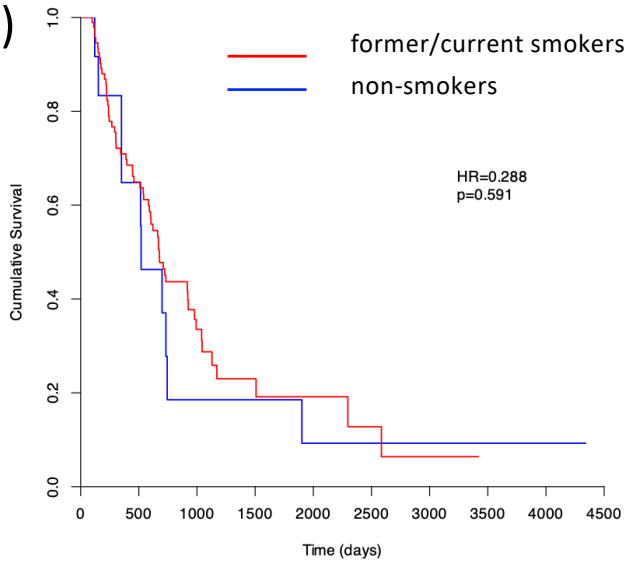
Characteristics	PD-L1 TPS 50-79% (N = 40)	PD-L1 TPS ≥80% (N = 60)	p-value for pairwise comparison (Mann-Whitney test)
Age - yr (at diagnosis)			0.428
Median	67	68	
Range	47-81	40-91	
Sex			0.955
Male - no. (%)	18 (45.0%)	26 (43.3%)	
Female - no. (%)	22 (55.0%)	34 (56.7%)	
Performance status			0.353
0 - no. (%)	7 (17.5%)	8 (13.3%)	
0-1 - no. (%)	0 (0%)	3 (5%)	
1 - no. (%)	20 (50%)	35 (58.3%)	
1-2 - no. (%)	0 (0%)	3 (5%)	
2 - no. (%)	8 (20%)	10 (16.7%)	
2-3 - no. (%)	3 (7.5%)	0 (0%)	
3 - no. (%)	2 (5.0%)	1 (1.7%)	
Smoking status			0.306
Current - no. (%)	22 (55.0%)	23 (38.3%)	
Former - no. (%)	12 (30.0%)	31 (51.7%)	
Never - no. (%)	6 (15.0%)	6 (10.0%)	
Histology			0.280
Squamous cell carcinoma - no. (%)	12 (30.0%)	13 (21.7%)	
Adenocarcinoma- no. (%)	25 (62.5%)	41 (68.3%)	
Adenosquamous - no. (%)	1 (2.5%)	1 (1.7%)	
Not specified - no. (%)	2 (5.0%)	5 (8.3%)	
EGFR mutation status			0.692
Positive - no. (%)	1 (2.5%)	1 (1.7%)	
Negative - no. (%)	25 (62.5%)	43 (71.7%)	
Unknown - no. (%)	14 (35.0%)	16 (26.7%)	
ALK translocation status			
Positive - no. (%)	0 (0%)	0 (0%)	1.000
Negative - no. (%)	25 (62.5%)	43 (71.7%)	
Unknown - no. (%)	15 (37.5%)	17 (28.3%)	
ROS-1 rearrangement status			1.000
Positive - no. (%)	0 (0%)	0 (0%)	
Negative - no. (%)	10 (25.0%)	19 (31.7%)	
Unknown - no. (%)	30 (75.0%)	41 (68.3%)	
Pembrolizumab therapy			0.101
First line (%)	34 (85%)	54 (85%)	
Second line (%)	6 (15%)	6 (15%)	
Stage			0.422
I - no. (%)	1 (2.5%)	1 (1.7%)	
II - no. (%)	3 (7.5%)	2 (3.3%)	
III - no. (%)	10 (25.0%)	14 (23.3.%)	
IV - no. (%)	26 (65.0%)	43 (71.7%)	
Radiotherapy before or within two months of immunotherapy			0.944
Radical - no. (%)	4 (10.0%)	6 (10.0%)	0.968
Palliative - no. (%)	12 (30.0%)	17 (28.3%)	0.961
Thoracic - no. (%)	4 (10.0%)	10 (16.7%)	
Extra-thoracic - no. (%)	8 (20.0%)	7 (11.7%)	

Mander *et al*, Supplemental Material, Appendix 1

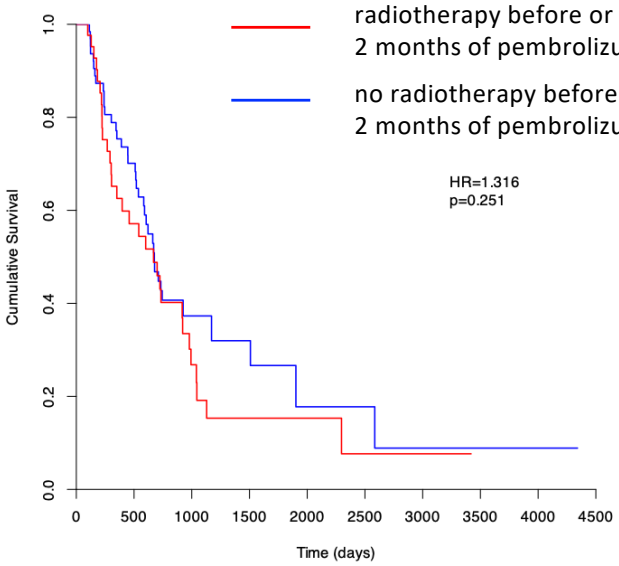
(A)



(B)



(C)



Mander *et al*, Supplemental Material, Appendix 2
Cox regression analysis

Variable	Hazard Ratio (HR)	Standard Error (SE)	p-value
PD-L1≥80% (yes/no)	1.178	0.286	0.568
Smoking status (yes/no)	2.040	0.408	0.081
Performance Status (PS)			**0.009**
PS(1)	0.074	0.747	0.000
PS(2)	0.523	0.857	0.449
PS(3)	0.133	0.661	0.002
PS(4)	0.279	0.982	0.193
PS(5)	0.137	0.695	0.004
(PS(6)	0.073	1.191	0.028
Stage			0.126
Stage(1)	0.616	1.032	0.639
Stage(2)	0.310	0.657	0.075
Stage(3)	0.504	0.370	0.064
Histology			0.827
Histology(1)	1.028	1.041	0.979
Histology(2)	1.237	1.071	0.843
Radiotherapy before of within 2 months of starting immunotherapy (yes/no)	0.478	0.322	**0.022**

BMJ Open

Pembrolizumab Monotherapy for Non-Small Cell Lung Cancer (NSCLC): Can Patient Stratification be Improved in the UK Tayside Population? A Retrospective Cohort Study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2023-076715.R2
Article Type:	Original research
Date Submitted by the Author:	12-Oct-2023
Complete List of Authors:	Mander, Emily ; University of Dundee, School of Medicine Merrick, Christopher ; NHS Tayside, Tayside Cancer Centre Nicholson, Hugh ; University of Dundee, School of Medicine Lord, Hannah ; NHS Tayside, Tayside Cancer Centre Ferguson, Michelle ; NHS Tayside, Tayside Cancer Centre Smith, Gillian; University of Dundee, School of Medicine
Primary Subject Heading:	Oncology
Secondary Subject Heading:	Patient-centred medicine
Keywords:	RADIOTHERAPY, CHEMOTHERAPY, Clinical Decision-Making, ONCOLOGY, Respiratory tract tumours < ONCOLOGY

SCHOLARONE™
Manuscripts

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

For peer review only

Manuscript for submission to BMJ Open, June 2023_Revised October 2023

Pembrolizumab Monotherapy for Non-Small Cell Lung Cancer (NSCLC): Can Patient Stratification be Improved in the UK Tayside Population? A Retrospective Cohort Study

Emily S Mander[1], Christopher B Merrick[2], Hugh A Nicholson[3], Hannah K Lord[2], Michelle J Ferguson[2], Gillian Smith[3]

[1]School of Medicine, University of Dundee; [2]Tayside Cancer Centre; [3]Division of Cellular & Systems Medicine, School of Medicine, University of Dundee, Ninewells Hospital & Medical School, Dundee DD1 9SY

Correspondence to: Dr Gillian Smith, Division of Cellular & Systems Medicine, School of Medicine, University of Dundee, Jacqui Wood Cancer Centre, Ninewells Hospital & Medical School, James Arrott Drive, Dundee DD1 8SY. Email: g.smith@dundee.ac.uk, ORCID 0000-0001-9288-7566.

For peer review only

Abstract

Objective: Pembrolizumab is a PD-1 inhibitor used to treat advanced NSCLC patients with PD-L1 tumour proportion score (TPS) ≥50%. Further TPS-based stratification has not been evaluated in the UK, although smoking-induced tumour mutational burden and the immunogenic effects of prior radiotherapy are suggested to improve response.

Aims: To investigate if PD-L1 TPS ≥80%, smoking status or radiotherapy before or within 2 months of treatment influenced progression-free survival (PFS) in NSCLC patients treated with pembrolizumab monotherapy.

Methods: PD-L1 TPS, smoking status and radiotherapy exposure were compared in NSCLC patients in NHS Tayside (n=100) treated with pembrolizumab monotherapy between 1st November 2017 and 18th February 2022. Survival estimates were compared using log rank analysis, and Cox proportional hazards analysis used to investigate the influence of potential confounding factors, including tumour stage and performance status.

Results: PFS was not significantly different (log rank hazard ratio (HR)=0.330, p=0.566) comparing patients with PD-L1 TPS 50-79% and PD-L1 TPS ≥80%. Smokers had significantly improved PFS (log rank HR=4.867, p=0.027), while patients receiving radiotherapy had significantly decreased PFS (log rank HR=6.649, p=0.012). A Cox regression model confirmed that both radiotherapy (p=0.022) and performance status (p=0.009) were independent negative predictors of PFS.

Conclusions: More rigorous PD-L1 TPS stratification did not influence survival outcomes. Smoking history improved PFS, although was not an independent response predictor, while radiotherapy and performance status independently influenced clinical response. We suggest that further stratification of PD-L1 TPS is not warranted, while performance status and radiotherapy treatment may be additional clinically useful biomarkers of response to pembrolizumab in NSCLC patients.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Strengths and Limitations of this Study

- Following Caldicott Guardian approval, 150 NSCLC patients were identified in a single centre in NHS Tayside, UK, following a diagnosis of non-small cell lung cancer and treatment with at least one cycle of pembrolizumab therapy, between November 2017 and February 18th, 2022.

- Patients (n=50) were excluded from the study if tumour PD-L1 TPS was unknown or <50%, they refused treatment, died after one cycle of pembrolizumab therapy, or pembrolizumab was prescribed in combination with chemotherapy.

- PD-L1 TPS for each tumour, assessed by immunohistochemistry, radiotherapy prescribing information and self-reported smoking data (never/current/former smokers) was obtained from clinical records.

- The influence of PD-L1 TPS (comparing TPS 50-79% and TPS TPS≥80%), radiotherapy and smoking status on PFS was assessed using Log-Rank analysis, and Cox proportional hazards models constructed to investigate whether significant conclusions were influenced by potential confounding variables, including performance status, stage and histology.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Introduction

Lung cancer, the third most common cancer in the UK and the principal cause of cancer mortality in both the UK and the USA (1) (2), is often diagnosed at late stage. Non-small cell lung cancer (NSCLC) is most commonly diagnosed, with a variety of histological types; adenocarcinoma (40%), squamous cell carcinoma (25%) and large cell carcinoma (10%) (3) (4). Advanced NSCLC (TNM stage III and IV) is treated with systemic anticancer therapy (SACT), as surgery is no longer possible (5). Chemotherapy offers poor survival outcomes in patients with advanced NSCLC, with a 1-year survival rate of around 30% (6). While subsets of NSCLCs have actionable targets including epidermal growth factor receptor (EGFR) mutations, anaplastic lymphoma kinase (ALK) translocations, and c-ROS oncogene 1 (ROS-1) rearrangements, the majority of NSCLCs do not express these oncogenic drivers (7).

Immune checkpoint inhibitors (ICI) targeting the programmed cell death protein-1/programmed cell death ligand-1 (PD-1/PD-L1) axis have revolutionised the treatment of advanced NSCLC, as they provide a stratified treatment option for patients with PD-L1 positive tumours but no other targetable mutations. PD-L1 expression is increased in NSCLC through aberrant signalling mechanisms resulting in T-cell inhibition which allows tumour cells to evade immune destruction (8) (9) (10).

Pembrolizumab is a monoclonal antibody which targets PD-1 on T-cells to disrupt the PD-1/PD-L1 axis (11) (12). Prescription of pembrolizumab in NSCLC is based on immunohistochemical assessment of % PD-L1 tumour proportion score (TPS) as a biomarker to stratify patients (13). In Scotland, Scottish Medicines Consortium (SMC) guidelines approve the use of pembrolizumab as first line monotherapy for advanced NSCLC in patients with PD-L1 TPS ≥50% with no EGFR mutations or ALK translocations. It is also licensed as second line monotherapy for patients with PD-L1 TPS ≥1% who have received at least one prior chemotherapy regime, and as first line treatment in combination with pemetrexed and platinum chemotherapy for advanced NSCLC patients with PD-L1 TPS <50%. Patients must not be eligible for alternative EGFR, ALK or ROS-1 targeted treatments as these can be targeted with specific inhibitors, such as the EGFR inhibitor gefitinib (14). The Keynote-010 clinical trial investigated superiority of pembrolizumab over docetaxel (OS HR 0·54, 95% CI 0·38–0·77, p=0.002, PFS HR 0·50, 95% CI 0·36–0·70, p=0.0001) (15) and confirmed improved response to pembrolizumab in patients with PD-L1 TPS ≥50%, while the Keynote-042 trial similarly reported improved pembrolizumab outcomes compared with investigator choice chemotherapy, when patients were stratified by TPS ≥50% (OS HR 0·69 (95% CI 0·56–0·85), p=0·0003, PFS HR 0·81 (95% CI 0·67–0·99), p=0·0170) (16).

While pembrolizumab monotherapy is a more effective treatment than chemotherapy for many NSCLC patients, it is associated with significant immune-related adverse effects, including thyroiditis, pneumonitis, colitis, nephritis, hypophysitis, hepatitis, encephalitis, myocarditis and severe cutaneous adverse reactions (SCARs) that can be severe and occasionally life-limiting (15) (17) (18). It is therefore important that the most appropriate patients are selected for pembrolizumab treatment. Disease response to pembrolizumab is routinely evaluated after two or three cycles of therapy and then every six to nine weeks thereafter. Response is evaluated radiologically, usually using CT scans, which are reported

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

using Response Evaluation Criteria in Solid Tumours (RECIST) criteria (7). Pembrolizumab therapy is associated with a rare treatment response known as pseudoprogression, where an initial increase in tumour burden is seen on imaging, with a subsequent reduction resulting in an overall decrease in tumour burden (19). The reported incidence of pseudoprogression in NSCLC patients treated with ICI is only 5% (20), although it is a significant clinical challenge as it is difficult to differentiate from true progression (20).

High mutational burden and associated molecular smoking signatures have been associated with increased efficacy of pembrolizumab therapy (21). Several studies have also linked cigarette smoking to high tumour PD-L1 expression (22) (23) (24) (25). For example, a prospective study in Canada involving 268 advanced NSCLC patients demonstrated that patients with PD-L1 TPS ≥50% who were smokers had a better response to anti-PD-1 immunotherapy than non-smokers. Objective response rate for current smokers was 36% compared to 26% in former smokers and 14% in non-smokers (p=0.02). Overall survival was also significantly increased in smokers compared to non-smokers. At 1-year post-diagnosis, 85.2% of current smokers were alive compared to 56.1% of former smokers and 42.6% of non-smokers (p=0.003) (26).

Radiotherapy can be used to treat NSCLC both palliatively and radically and has been hypothesised to have an immunostimulatory effect (27) (28), resulting from the release of damage-associated molecular pattern molecules (DAMPs) following tumour cell destruction by radiation. DAMPs activate dendritic cells which trigger the immune system to mount a specific T-cell response (29) (30), resulting in an “abscopal effect”, where tumour sites distant from the location of radiotherapy start to regress (31).

A secondary analysis of the Keynote-001 clinical trial of pembrolizumab in NSCLC investigated the effects of radiotherapy prior to pembrolizumab monotherapy and found that patients who had received prior radiotherapy had a significantly increased median progression-free (4.4 months compared to 2.1 months in the group who did not receive prior radiotherapy (p=0.019)) and overall survival (10.7 months compared to 5.3 months in patients who did not receive prior radiotherapy (p=0.026)). At 6 months progression-free survival was 49% in the prior radiotherapy group compared to 23% in patients that did not receive prior radiotherapy (p=0.019) (32).

The PEMBRO-RT Phase II clinical trial was designed to investigate whether stereotactic ablative radiotherapy (SABR) prior to pembrolizumab therapy resulted in enhanced treatment response in metastatic NSCLC, regardless of PD-L1 expression. 76 patients were randomised in a 1:1 ratio to receive either pembrolizumab monotherapy (control group) or SABR prior to pembrolizumab (experimental group). Median progression-free survival was 6.6 months in the SABR group compared to only 1.9 months in the control group, although this difference was not statistically significant (p=0.19). Similarly, median overall survival was 15.9 months in the SABR group compared to 7.6 months in the no radiotherapy group (p=0.16) (33).

As well as PD-L1 TPS, smoking and radiotherapy there are other important modifiers of outcome to consider for all cancer patients, including performance status and the stage and histology. Performance status is a measure of the functional status of a patient and is assessed

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

using the Eastern Cooperative Oncology Group Score (ECOG) Performance Status Scale, with scores from zero to five, where zero indicates no functional deficit and 5 confirms that the patient is deceased (34). Several studies have suggested that patients with performance status ≥2 have worse survival outcomes following pembrolizumab treatment than patients with performance status 0-1 (35) (36) (37).

This study aimed to investigate whether pembrolizumab patient selection could be refined by further sub-division of PD-L1 expression thresholds, and whether previous data describing a positive association of smoking on progression-free survival in NSCLC patients on pembrolizumab therapy was seen in the UK Tayside population. Based on current literature reporting potential immunostimulatory effects of radiotherapy, we also aimed to investigate the influence of radiotherapy on progression-free survival in NSLCC patients prescribed pembrolizumab in routine clinical practice, outwith a controlled clinical trial setting.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Methods

1. Study Approval

Caldicott Guardian Approval was received to allow collection of confidential NSCLC patient information in NHS Tayside.

2. Patient Selection

Study data was collected from NHS computers in Ward 32 Oncology, Ninewells Hospital & Medical School, Dundee between 31st January 2022 and 18th February 2022, with further follow up data collection from 5th January 2023 to 19th February 2023. All patient data was anonymised before inclusion in the study. 150 NSCLC patients were identified from the NHS Tayside oncology database following a diagnosis of non-small cell lung cancer and treatment with at least one cycle of pembrolizumab therapy between November 2017 and February 18th, 2022. Patients were excluded from the study if tumour PD-L1 TPS was unknown (n=1) or <50% (n=9), they refused treatment (n=1), died after one cycle of pembrolizumab therapy when radiological progression data was not available (n=7), or pembrolizumab was prescribed in combination with chemotherapy (triple therapy, n=23) (Figure 1, Supplementary Table 1). Demographic information for all patients, including age, sex, performance status, tumour histology, tumour stage and EGFR, ALK and ROS-1 status was obtained from the Chemocare database, ICE and Clinical Portal.

3. PD-L1 Expression Data

PD-L1 TPS for each tumour, assessed by immunohistochemistry, was obtained from pathology reports or reports from Tayside Lung Cancer Multi-disciplinary Team Meetings (MDTs), obtained from the ICE database. Patients were then stratified into two groups: PD-L1 TPS 50-79% and PD-L1 TPS ≥80%.

4. Radiotherapy Data

Oncology records, accessed through the Clinical Portal database, were used to document the date, type and location of any radiotherapy given. Patients were initially stratified into two groups: those who received radiotherapy at any time before or within two months of immunotherapy, and those who did not receive radiotherapy before or within two months of immunotherapy. Patients were then further sub-divided by palliative or radical radiotherapy, with patients receiving palliative radiotherapy further divided into two subgroups based on radiotherapy location (thoracic or extra thoracic).

5. Smoking Data

Self-reported smoking status was obtained from medical records using the Clinical Portal database. Patients were first divided into two groups: patients who had ever smoked and patients who had never smoked. Patients who had smoked were then further divided into current smokers and former smokers.

6. Study Outcomes

Due to the retrospective nature of the study, many patients went on to receive other forms of systemic anticancer therapy (SACT), so there were many potential confounding variables that could influence overall survival. Therefore, consistent with other similar retrospective

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

cohort studies involving immunotherapy in NSCLC, progression-free survival (PFS) was used as the primary outcome of the study. PFS was calculated as the time in days from the start of cycle one of pembrolizumab therapy to the date of radiological disease progression. Treatment response CT scans were carried out every six to nine weeks in this patient cohort. Overall survival, assessed as a secondary endpoint, was calculated as the time in days between the date of diagnosis and the date of death or census end point (February 18th, 2022).

7. Statistical Analysis

Statistical analysis was carried out using version 27 of the SPSS statistics programme (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0, Armonk, NY: IBM Corp). Baseline patient demographics were compared in patients with PD-L1 50-79% and PD-L1≥80% using Mann-Whitney tests for non-parametric data. Progression-free and overall survival were assessed using Log-Rank analysis, with Kaplan-Meier Survival Plots created using the ggplot2 and survival packages and Cairo function in the open-source R programming environment Version 2023.03.1+446 (38). If the Kaplan-Meier Plots produced significant results, further Cox proportional hazards models were constructed in SPSS to investigate whether significant conclusions were influenced by potential confounding variables, including performance status, stage and histology.

8. Patient and Public Involvement Statement

Patients or the public were not involved in the design, conduct, reporting or dissemination plans of our research.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Results

1. Patient Demographics

150 patients were initially assessed for inclusion in the study, however final analysis was carried out on 100 patients as 50 patients did not meet the inclusion criteria - 1 patient refused treatment, 9 patients did not have a sample available for PD-L1 testing, PD-L1 TPS was not quantified in 1 patient, 23 patients received triple therapy, 9 patients had PD-L1 TPS <50% and 7 patients died after one cycle of pembrolizumab (Figure 1). Patient demographics are further summarised in Supplementary Table 1.

2. Does PD-L1 TPS 50-79% in comparison to ≥80% influence PFS or OS?

To investigate if stratification of NSCLC patients for pembrolizumab treatment could be further refined by very high PD-L1 TPS (≥80%), patients were separated into two groups; PD-L1 TPS 50-79% and PD-L1 TPS ≥80%, with PD-L1 TPS assessed as described in Methods. There was no significant difference comparing progression-free survival in NSCLC patients with PD-L1 TPS 50-79% and those with PD-L1 TPS ≥80% (HR=0.330, p=0.566) (Figure 2). Similarly, there was no significant difference in overall survival comparing patients with PD-L1 TPS 50-79% and those with PD-L1 TPS ≥80% (HR=0.120, p=0.729) (Supplementary Figure 1A). In additional exploratory analysis, we increased the PD-L1 TPS threshold to 90%, comparing patients with PD-L1 TPS 50-89% and PD-L1 TPS ≥90%, but again found no significant differences in PFS or OS (data not shown).

3. Does smoking history influence survival outcomes in NSCLC patients prescribed pembrolizumab?

To investigate if smoking status had a significant impact on PFS, patients were sub-divided according to smoking status, as described in Methods. Patients who were smokers (defined as current or former smokers) had significantly longer progression-free survival compared to patients who were non-smokers (HR=4.867, p=0.027) (Figure 3A). Patients were then further subdivided into current smokers, former smokers and non-smokers, with no significant differences in PFS in current smokers and former smokers (HR=5.248, p=0.073) (Figure 3B). In contrast, no significant difference in overall survival was seen in patients who were smokers and those who were non-smokers (HR=0.288, p=0.591) (Supplementary Figure 1B).

4. Does prior radiotherapy treatment influence survival outcomes in NSCLC patients prescribed pembrolizumab?

To investigate the influence of radiotherapy on progression-free survival, patients were categorised based on whether or not they had received radiotherapy before or within two months of pembrolizumab monotherapy, as described in Methods. In contrast to published data, patients who received radiotherapy had significantly decreased progression-free survival compared to patients who did not receive radiotherapy (HR=6.254, p=0.012) (Figure 4). Similar to our smoking data, there was no significant difference in overall survival between patients who received radiotherapy before or within two months of pembrolizumab monotherapy and those who did not (HR=1.316, p=0.251) (Supplementary Figure 1C).

For peer review only

A Cox Regression Model was then used to investigate whether the significant smoking and radiotherapy associations reported above were modified by potential confounding factors including performance status, tumour stage and histology. Cox regression analysis confirmed that radiotherapy at any point before or within two months of pembrolizumab monotherapy (p=0.022) and performance status (0.009), but not stage (p=0.126), histology (p=0.827), PD-L1 TPS (p=0.568) or smoking status (p=0.081) were independent predictors of PFS in NSCLC patients treated with pembrolizumab (Supplementary Table 2).

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Discussion

Approval of pembrolizumab has revolutionised the treatment of advanced and metastatic NSCLC, although treatment is expensive and patient selection limited to immunohistochemical assessment of tumour proportion score (TPS), with patients with PD-L1 TPS ≥50% currently eligible for treatment. To investigate whether more rigorous TPS stratification might influence treatment response in routine clinical practice, we compared PD-L1 TPS 50-79% and PD-L1 TPS ≥80% in a cohort of unselected NSCLC patients treated in a single centre, and further investigated whether clinical outcomes were influenced by smoking, previous radiotherapy exposure or could simply be predicted by performance status.

We first investigated whether further stratification of PD-L1 TPS might lead to improved clinical outcomes in NSCLC patients. For consistency with previous reports, we used PFS as our primary and OS as secondary analysis endpoint in order to limit additional sources of variation, as many patients received additional SACT following disease progression on pembrolizumab monotherapy. We found no significant difference in either progression-free (HR=0.330, p=0.566) or overall (HR=0.120, p=0.729) survival, comparing patients with PD-L1 TPS 50-79% and PD-L1 TPS ≥80% and in further analysis increasing the PD-L1 TPS threshold to 90% , suggesting that further TPS-based patient stratification may not be warranted. We chose to initially exclude 7 patients from our analysis as they died following 1 cycle of pembrolizumab, when it had not been possible to investigate disease progression by CT scan – to ensure that exclusion of these patients had not inadvertently influenced our survival analysis, we confirmed that our OS data was similar in the extended dataset. Our data contrasts with the results of an American retrospective study (n=187 patients), which reported an association of PD-L1 TPS ≥90% with significantly improved PFS (14.5 months vs 4.1 months, HR=0.50, p<0.01) (39). However, similar to our own data, a retrospective cohort study in Japan (n=149 patients), comparing PFS in patients with PD-L1 TPS 50-89% and 90-100% reported no significant difference in progression-free survival (HR=0.78, p=0.34). PFS in the Japanese study at 120 days was 64.4% in PD-L1 TPS 50-89% patients and 63.0% in PD-L1 TPS 90-100% patients (HR=1.03, p=0.09) (40), similar to our own data which reports PFS of 70% at 120 days in the PD-L1 50-79% group and 76% in the PD-L1 ≥80% group (p=0.566). Both the American and Japanese studies used higher (≥90%) PD-L1 TPS to stratify patients, and it is important to note that the American study reported TPS using four different antibodies due to differences in practice between institutions. This observation highlights the limitations of PD-L1 as a quantitative biomarker. Although testing is standardised across Scotland, using the same Dako 22C3 antibody reported in the early Keynote trials (41) (17), PD-L1 TPS is routinely reported following expert pathologist assessment of immunohistochemical staining, with associated inherent variation between centres and reporting pathologists (42). Tumour heterogeneity at diagnosis is additionally recognised to significantly influence PD-L1 expression (43), and it is likely that expression varies further during disease progression and treatment. Despite these limitations, baseline PD-L1 TPS assessed from the initial diagnostic biopsy is currently routinely used to inform patient selection for immunotherapy. We highlight the need in future studies to develop more quantitative methods for PD-L1 assessment, to facilitate more rigorous evaluation of the potential of TPS as a predictive and prognostic biomarker.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Our data initially confirmed previous reports (23) (24) (44), suggesting that patients who were current or former smokers had significantly longer PFS than non-smokers (HR=4.867, p=0.027). Importantly, PFS in current smokers and former smokers was not significantly different (HR=5.248, p=0.073), suggesting that any smoking history has the potential to modify pembrolizumab response. Consistent with our data, a recent meta-analysis investigating the impact of smoking status on targeted therapy in NSCLC in Phase III clinical trials reported that smokers had significantly extended PFS following immune checkpoint inhibitor treatment (HR=1.81, p=0.004) (44), with additional meta-analyses reporting similar conclusions (23) (24). It is also important to note, however, that our extended Cox regression analysis did not confirm smoking history as an independent predictor of pembrolizumab response in NSCLC, and that the influence of confounding factors has not always been previously reported. Although it is logical that smoking may increase tumour mutation burden (TMB) and, as a consequence, increase immunogenicity and improve response to immunotherapy, it is important to acknowledge that TMB has not been routinely assessed in significant numbers of patients outwith the clinical trial setting, and that results from some previous studies do not support this hypothesis (21). The use of smoking status as a biomarker for pembrolizumab response additionally raises important ethical issues as smoking cessation is an important part of the clinical management of lung cancer, as it improves outcomes and reduces the risk of the development of further cancers (45) (46) and other diseases associated with smoking such as cardiovascular disease and chronic obstructive pulmonary disease (COPD) (47). Further, in this and previous studies, patients were identified as smokers or non-smokers based on self-reported smoking history. Verification of smoking status, for example using biochemical confirmation of serum cotinine levels, is recommended but is challenging outwith the clinical trial setting (48), and self-reported smoking history is more likely to be under rather over-represented, in turn under-estimating pembrolizumab response predictions in smokers. Serum cotinine has been successfully used to confirm self-reported smoking status to identify eligible patients for lung cancer screening (48) and can also be used in patients using electronic cigarettes containing nicotine (49) (50). We highlight the need to include more quantitative and objective assessment of smoking history in future studies to investigate whether the modifying effect on ICI response in NSCLC patients is dose-dependent and whether smoking status and TPS are independent risk modifiers.

Our analysis suggests that NSCLC patients receiving radiotherapy before or within two months of pembrolizumab monotherapy had significantly decreased PFS compared to patients who did not receive radiotherapy (HR=6.254, p=0.012), in contrast to the findings of the Keynote-001 clinical trial (32) which reported that radiotherapy increased the efficacy of immunotherapy, possibly due to the abscopal effect (51). Further studies, however, including a retrospective multicentre study evaluating the effects of palliative radiotherapy before or within three months of anti-PD-1 therapy reported no significant difference in PFS, comparing patients who had received radiotherapy and those who had not (3.2 months vs 2.0 months, p=0.515) (52), while the PEMBRO-RT trial also reported no significant difference in PFS in patients who received SABR prior to pembrolizumab therapy and those who did not (1.9 months vs 6.6 months, p=0.19), although the data suggested that the possible benefit of prior radiotherapy should be further investigated in a larger dataset (53). We acknowledge that patients receiving radiotherapy within 2 months of pembrolizumab in our study may have had more advanced disease, or may have progressed more quickly, although tumour stage at diagnosis was not independently predictive of PFS.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

In contrast to previously reported clinical trial data, the majority of patients in the current study received palliative radiotherapy (usually 8Gy in 1 fraction or 20Gy in 5 fractions (54)) rather than SABR. It is therefore possible that palliative radiotherapy does not potentiate immunogenicity in NSCLC patients, as most previous literature reports on the influence of higher dose SABR on immunotherapy outcomes (55). As many of our study patients had symptomatic metastases, it is also possible that the modifying effect of radiotherapy we report, while independently predictive of survival outcomes, may simply represent a surrogate marker for performance status. Many NSCLC patients are additionally prescribed steroids, either to alleviate tumour compression or the side effects of immunotherapy. Steroid use is known to suppress the immune system and may therefore further modify responses to both radiotherapy and immunotherapy (56). We highlight the need to investigate the potential modifying effect of steroid prescription in future studies, and also the potential modifying effect of radiotherapy and pembrolizumab scheduling, as tumour repopulation post radiotherapy may further influence pembrolizumab response (57) (58). It is also important to ensure that CT scan reporting is standardised as far as is practicable in routine clinical practice. In the Keynote-024 clinical trial, for example, CT scans were all reported according to RECIST criteria, by a radiologist independent from the trial (8). While undoubtedly increasing the accuracy of clinical response estimates, greater variation in CT reporting in routine clinical practice is inevitable, even in a single centre. Radiological response assessment is particularly important following immunotherapy treatment due to pseudo-progression, where an initial apparent increase in tumour burden due to accumulation of immune cells causing an inflammatory response result in enlargement of neoplastic lesions (19), followed by subsequent regression (59), and is difficult to differentiate from true disease progression through initial imaging (20) (60). To address this relatively rare complication (incidence <6% in NSCLC patients), revised Response Evaluation Criteria in Solid Tumours (RECIST) guidelines, iRECIST, were developed in 2017 to improve reporting in immunotherapy clinical trials (61).

Importantly, despite these acknowledged sources of variation in biomarker and radiological assessment, our data highlights that performance status is an independent predictor of PFS (p=0.009). We assessed outcomes in all NSCLC patients treated with pembrolizumab (performance status 0-3), in contrast to more restricted clinical trials where, for example, only patients with performance status 0-1 were included in the Keynote-024 clinical trial (17), and the PePS2 single arm Phase 2 trial evaluated pembrolizumab response in patients with PS ≥2 (62). Consistent with our findings, several previous studies have reported that patients with PS ≥2 have reduced survival outcomes (35) (36) (37), while a recent Italian multicentre retrospective study confirmed that performance status was an independent predictor of poor clinical outcome (63).

In conclusion, therefore, our data confirms that more rigorous stratification of NSCLC patients by PD-L1 TPS did not influence survival outcomes. Smoking status (current or previous smoker) significantly improved PFS, although was not an independent predictor of survival. In contrast, radiotherapy treatment at any point before or within two months of pembrolizumab therapy independently adversely influenced PFS, and performance status was shown to be an independent predictor of clinical response. We suggest that further stratification of PD-L1 TPS may not be warranted, the modifying effects of radiotherapy

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

require further investigation in carefully controlled future studies, and performance status in addition to the currently used PD-L1 TPS ≥50% may be a clinically useful biomarker of response to pembrolizumab in NSCLC patients.

Footnotes

- **Contributors** ESM: conceptualisation, methodology, investigation, writing (original draft), visualisation; HAN: investigation, writing (original draft), visualisation; CJM and HKL: investigation, writing (review and editing); MJF and GS: conceptualisation, methodology, investigation, writing (original draft), visualisation, supervision. As guarantor, GS accepts full responsibility for the work, had access to the data and controlled the decision to publish.
- **Ethics statement** This is a retrospective cohort study of anonymised clinical data and does not require ethics approval. Caldicott Guardian Approval was received to allow collection of confidential NSCLC patient information in NHS Tayside.
- **Funding** The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors. This research was performed as part of an intercalated University of Dundee degree in Genetics, Cancer & Personalised Medicine.
- **Presentation** Some of this research has been previously presented at the CRUK Lung Cancer Conference 2022, Manchester, UK.
- **Competing interests** None declared.
- **Patient and public involvement** Patients and/or the public were not involved in the design, conduct, reporting or dissemination plans of this research.
- **Provenance and peer review** Not commissioned; externally peer reviewed.
- **Supplemental material** This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

Peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

References:

1. Cancer Research UK. Lung Cancer Statistics, 2018 [Available from: https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/lung-cancer.]
2. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. CA Cancer J Clin. 2022;72(1):7-33.
3. NHS. Lung Cancer, 2019 [Available from: https://www.nhs.uk/conditions/lung-cancer/.]
4. National Cancer Institue. Non-Small Cell Lung Cancer Treatment: National Cancer Institute; 2022 [Available from: https://www.cancer.gov/types/lung/hp/non-small-cell-lung-treatment-pdq#_359.]
5. Molina JR, Yang P, Cassivi SD, Schild SE, Adjei AA. Non-Small Cell Lung Cancer: Epidemiology, Risk Factors, Treatment, and Survivorship. Mayo Clin Proc. 2008;83(5):584-94.
6. Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, et al. Comparison of Four Chemotherapy Regimens for Advanced Non–Small-Cell Lung Cancer. New England Journal of Medicine. 2002;346(2):92-8.
7. Planchard D, Popat S, Kerr K, Novello S, Smit EF, Faivre-Finn C, et al. Metastatic Non-Small Cell Lung Cancer: ESMO Clinical Practice Guidelines for Diagnosis, Treatment and Follow-up. Annals of Oncology. 2018;29(Supplement_4):iv192-iv237.
8. Ghosh C, Luong G, Sun Y. A Snapshot of the PD-1/PD-L1 Pathway. Journal of Cancer. 2021;12(9):2735-46.
9. Jiang Y, Chen M, Nie H, Yuan Y. PD-1 and PD-L1 in Cancer Immunotherapy: Clinical Implications and Future Considerations. Human Vaccines & Immunotherapeutics. 2019;15(5):1111-22.
10. Hanahan D, Weinberg RA. Hallmarks of Cancer: The Next Generation. Cell. 2011;144(5):646-74.
11. Kwok G, Yau TC, Chiu JW, Tse E, Kwong YL. Pembrolizumab (Keytruda). Hum Vaccin Immunother. 2016;12(11):2777-89.
12. BNF. Pembrolizumab | Drug | BNF content published by NICE: NICE; [Available from: https://bnf.nice.org.uk/drug/pembrolizumab.html.]
13. De Marchi P, Leal LF, Duval da Silva V, da Silva ECA, Cordeiro de Lima VC, Reis RM. PD-L1 Expression by Tumor Proportion Score (TPS) and Combined Positive Score (CPS) are Similar in Non-Small Cell Lung Cancer (NSCLC). Journal of Clinical Pathology. 2021;74(11):735-40.
14. SMC. Pembrolizumab (Keytruda): Scottish Medicines Consortium; [Available from: https://www.scottishmedicines.org.uk/medicines-advice/pembrolizumab-keytruda-fullsubmission-123917/.]
15. Herbst RS, Baas P, Kim DW, Felip E, Pérez-Gracia JL, Han JY, et al. Pembrolizumab Versus Docetaxel for Previously Treated, PD-L1-Positive, Advanced Non-Small-Cell Lung Cancer (KEYNOTE-010): A Randomised Controlled trial. Lancet. 2016;387(10027):1540-50.
16. Gandhi L, Rodríguez-Abreu D, Gadgeel S, Esteban E, Felip E, De Angelis F, et al. Pembrolizumab Plus Chemotherapy in Metastatic Non-Small-Cell Lung Cancer. N Engl J Med. 2018;378(22):2078-92.
17. Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, et al. Pembrolizumab Versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. N Engl J Med. 2016;375(19):1823-33.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

18. EMA. Keytruda - Summary of Product Characteristics [Available from: https://www.ema.europa.eu/en/documents/product-information/keytruda-epar-product-information_en.pdf.]
19. Jia W, Gao Q, Han A, Zhu H, Yu J. The Potential Mechanism, Recognition and Clinical Significance of Tumor Pseudoprogression after Immunotherapy. Cancer Biol Med. 2019;16(4):655-70.
20. Park HJ, Kim KW, Pyo J, Suh CH, Yoon S, Hatabu H, et al. Incidence of Pseudoprogression During Immune Checkpoint Inhibitor Therapy for Solid Tumors: A Systematic Review and Meta-Analysis. Radiology. 2020;297(1):87-96.
21. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Mutational Landscape Determines Sensitivity to PD-1 Blockade in Non-Small Cell Lung Cancer. Science. 2015;348(6230):124-8.
22. Norum J, Nieder C. Tobacco Smoking and Cessation and PD-L1 Inhibitors in Non-Small Cell Lung Cancer (NSCLC): A Review of the Literature. ESMO Open. 2018;3(6):e000406.
23. Zhao W, Jiang W, Wang H, He J, Su C, Yu Q. Impact of Smoking History on Response to Immunotherapy in Non-Small-Cell Lung Cancer: A Systematic Review and Meta-Analysis. Front Oncol. 2021;11:703143.
24. Lee KWC, Lord SJ, Kasherman L, Marschner I, Stockler M, Gralla R, et al. The Impact of Smoking on the Effectiveness of Immune Checkpoint Inhibitors — A Systematic Review and Meta-analysis. Acta Oncologica. 2020;59(1):96-100.
25. Abdel-Rahman O. Smoking and EGFR Status May Predict Outcomes of Advanced NSCLC Treated with PD-(L)1 Inhibitors Beyond First Line: A Meta-analysis. The Clinical Respiratory Journal. 2018;12(5):1809-19.
26. Li JJN, Karim K, Sung M, Le LW, Lau SCM, Sacher A, et al. Tobacco Exposure and Immunotherapy Response in PD-L1 Positive Lung Cancer Patients. Lung Cancer. 2020;150:159-63.
27. SIGN. Management of Lung Cancer. 2014.
28. Reynders K, Illidge T, Siva S, Chang JY, De Ruysscher D. The Abscopal Effect of Local Radiotherapy: Using Immunotherapy to Make a Rare Event Clinically Relevant. Cancer Treatment Reviews. 2015;41(6):503-10.
29. Bhalla N, Brooker R, Brada M. Combining Immunotherapy and Radiotherapy in Lung Cancer. Journal of Thoracic Disease. 2018;10(S13):S1447-S60.
30. Corke L, Sacher A. New Strategies and Combinations to Improve Outcomes in Immunotherapy in Metastatic Non-Small-Cell Lung Cancer. Current Oncology. 2021;29(1):38-55.
31. Turgeon GA, Weickhardt A, Azad AA, Solomon B, Siva S. Radiotherapy and Immunotherapy: A Synergistic Effect in Cancer Care. Medical Journal of Australia. 2019;210(1):47-53.
32. Shaverdian N, Lisberg AE, Bornazyan K, Veruttipong D, Goldman JW, Formenti SC, et al. Previous Radiotherapy and the Clinical Activity and Toxicity of Pembrolizumab in the Treatment of Non-Small-Cell Lung Cancer: A Secondary Analysis of the KEYNOTE-001 Phase 1 Trial. The Lancet Oncology. 2017;18(7):895-903.
33. Welsh J, Menon H, Chen D, Verma V, Tang C, Altan M, et al. Pembrolizumab With or Without Radiation Therapy for Metastatic Non-Small Cell Lung Cancer: A Randomized Phase I/II Trial. Journal for ImmunoTherapy of Cancer. 2020;8(2):e001001.
34. ECOG-ACRIN Cancer Research Group. ECOG Peformance Status Scale [Available from: https://ecog-acrin.org/resources/ecog-performance-status/.]

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

35. Tamiya M, Tamiya A, Hosoya K, Taniguchi Y, Yokoyama T, Fukuda Y, et al. Efficacy and Safety of Pembrolizumab as First-Line Therapy in Advanced Non-Small Cell Lung Cancer With At Least 50% PD-L1 Positivity: A Multicenter Retrospective Cohort Study (HOPE-001). Investigational new drugs. 2019;37(6):1266-73.
36. Sehgal K, Gill RR, Widick P, Bindal P, McDonald DC, Shea M, et al. Association of Performance Status With Survival in Patients With Advanced Non-Small Cell Lung Cancer Treated With Pembrolizumab Monotherapy. JAMA Netw Open. 2021;4(2):e2037120.
37. Addeo A, Metro G, Signorelli D, Economopoulou P, Roila F, Banna GL, et al. Poor Performance Status and Front-Line Pembrolizumab in Advanced Non-Small-Cell Lung Cancer (NSCLC) Patients with PD-L1>50%. Journal of Clinical Oncology. 2020;38(15_suppl):e21651-e.
38. R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
39. Aguilar EJ, Ricciuti B, Gainor JF, Kehl KL, Kravets S, Dahlberg S, et al. Outcomes to First-Line Pembrolizumab in Patients with Non-Small-Cell Lung Cancer and Very High PD-L1 Expression. Ann Oncol. 2019;30(10):1653-9.
40. Edahiro R, Kanazu M, Kurebe H, Mori M, Fujimoto D, Taniguchi Y, et al. Clinical outcomes in Non-Small Cell Lung Cancer Patients with an Ultra-High Expression of Programmed Death Ligand-1 Treated Using Pembrolizumab As A First-Line Therapy: A Retrospective Multicenter Cohort Study In Japan. PloS one. 2019;14(7):e0220570-e.
41. Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, et al. Pembrolizumab for the Treatment of Non–Small-Cell Lung Cancer. New England Journal of Medicine. 2015;372(21):2018-28.
42. Ming Sound Tsao KMK, Sanja Dacic, Yasushi Yatabe, Fred R. Hirsch. IASLC Atlas of PD-L1 Immunohistochemistry Testing in Lung Cancer: International Association for the Study of Lung Cancer.
43. Munari E, Mariotti FR, Quatrini L, Bertoglio P, Tumino N, Vacca P, et al. PD-1/PD-L1 in Cancer: Pathophysiological, Diagnostic and Therapeutic Aspects. International Journal of Molecular Sciences. 2021;22(10):5123.
44. Li X, Huang C, Xie X, Wu Z, Tian X, Wu Y, et al. The Impact of Smoking Status on the Progression-Free Survival of Non-Small Cell Lung Cancer Patients Receiving Molecularly Target Therapy or Immunotherapy Versus Chemotherapy: A Meta-Analysis. Journal of Clinical Pharmacy and Therapeutics. 2021;46(2):256-66.
45. Luo SJ, Choi E, Aredo JV, Wilkens LR, Tammemägi MC, Le Marchand L, et al. Smoking Cessation After Lung Cancer Diagnosis and the Risk of Second Primary Lung Cancer: The Multiethnic Cohort Study. JNCI Cancer Spectr. 2021;5(5).
46. Caini S, Del Riccio M, Vettori V, Scotti V, Martinoli C, Raimondi S, et al. Quitting Smoking At or Around Diagnosis Improves the Overall Survival of Lung Cancer Patients: A Systematic Review and Meta-Analysis. J Thorac Oncol. 2022;17(5):623-36.
47. NHS. What Are The Health Risks Of Smoking? : NHS; 2018 [Available from: https://www.nhs.uk/common-health-questions/lifestyle/what-are-the-health-risks-of-smoking/.]
48. Liu B, Henschke CI, Flores RM, Taioli E. Serum Cotinine Verification of Self-Reported Smoking Status Among Adults Eligible for Lung Cancer Screening in the 1999-2018 National Health and Nutrition Examination Survey. Lung Cancer. 2020;144:49-56.
49. Vélez de Mendizábal N, Jones DR, Jahn A, Bies RR, Brown JW. Nicotine and Cotinine Exposure from Electronic Cigarettes: A Population Approach. Clin Pharmacokinet. 2015;54(6):615-26.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

50. Rapp JL, Alpert N, Flores RM, Taioli E. Serum Cotinine Levels and Nicotine Addiction Potential of E-Cigarettes: An NHANES Analysis. Carcinogenesis. 2020;41(10):1454-9.
51. Theelen WS, De Jong MC, Baas P. Synergizing Systemic Responses by Combining Immunotherapy with Radiotherapy in Metastatic Non-Small Cell Lung Cancer: The Potential of the Abscopal Effect. Lung Cancer. 2020;142:106-13.
52. Samuel E, Lie G, Balasubramanian A, Hiong A, So Y, Voskoboynik M, et al. Impact of Radiotherapy on the Efficacy and Toxicity of anti-PD-1 Inhibitors in Metastatic NSCLC. Clinical Lung Cancer. 2021;22(3):e425-e30.
53. Theelen WSME, Peulen HMU, Lalezari F, Van Der Noort V, De Vries JF, Aerts JGJV, et al. Effect of Pembrolizumab After Stereotactic Body Radiotherapy vs Pembrolizumab Alone on Tumor Response in Patients With Advanced Non–Small Cell Lung Cancer. JAMA Oncology. 2019;5(9):1276.
54. Lord H. Clinical Management Protocol - Non-Surgical Management of Non-Small Cell Lung Cancer: NHS Tayside; 2015 [Available from: https://www.nhstaysideadtc.scot.nhs.uk/tapg%20html/Specialist%20Lists/Oncology-Haematology/Protocols/Nonsmall%20cell%20lung%20prot.pdf.]
55. Damen PJJ, Verhoeff JJC. Efficacy Of Stereotactic Ablative Radiotherapy (SABR) During Anti-PD-1 In Oligoprogressive Non-Small Cell Lung Cancer And Melanoma-A Prospective Multicenter Observational Study Pointing Out New Unmet Needs. Transl Cancer Res. 2023;12(3):688-91.
56. Petrelli F, Signorelli D, Ghidini M, Ghidini A, Pizzutilo EG, Ruggieri L, et al. Association of Steroids Use With Survival In Patients Treated With Immune Checkpoint Inhibitors: A Systematic Review And Meta-Analysis. Cancers (Basel). 2020;12(3).
57. Ng WL, Huang Q, Liu X, Zimmerman M, Li F, Li CY. Molecular Mechanisms Involved In Tumor Repopulation After Radiotherapy. Transl Cancer Res. 2013;2(5):442-8.
58. Pajonk F, Vlashi E, McBride WH. Radiation Resistance Of Cancer Stem Cells: The 4 R's Of Radiobiology Revisited. Stem Cells. 2010;28(4):639-48.
59. Ma Y, Wang Q, Dong Q, Zhan L, Zhang J. How to Differentiate Pseudoprogression from True Progression in Cancer Patients Treated with Immunotherapy. Am J Cancer Res. 2019;9(8):1546-53.
60. Zhou L, Zhang M, Li R, Xue J, Lu Y. Pseudoprogression and Hyperprogression In Lung Cancer: A Comprehensive Review Of Literature. Journal of Cancer Research and Clinical Oncology. 2020;146(12):3269-79.
61. Seymour L, Bogaerts J, Perrone A, Ford R, Schwartz LH, Mandrekar S, et al. iRECIST: Guidelines For Response Criteria For Use in Trials Testing Immunotherapeutics. Lancet Oncol. 2017;18(3):e143-e52.
62. Middleton G, Brock K, Savage J, Mant R, Summers Y, Connibear J, et al. Pembrolizumab in Patients with Non-Small-Cell Lung Cancer of Performance Status 2 (PePS2): A Single Arm, Phase 2 Trial. The Lancet Respiratory Medicine. 2020;8(9):895-904.
63. Cortellini A, Tiseo M, Banna GL, Cappuzzo F, Aerts JGJV, Barbieri F, et al. Clinicopathologic Correlates of First-Line Pembrolizumab Effectiveness in Patients with Advanced NSCLC and a PD-L1 Expression of ≥ 50. Cancer Immunology, Immunotherapy. 2020;69(11):2209-21.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Figure Legends

Figure 1: Patient Selection and Demographics
150 NSCLC patients were initially identified in NHS Tayside, between 31st January 2022 and 18th February 2022. 50 patients were excluded from the study as they failed to meet the inclusion criteria, for the reasons indicated. Patients were classified as smokers if they were current or ex-smokers, based on self-reported smoking status.

Figure 2: Further patient stratification by PD-L1 TPS does not influence PFS
Log-Rank analysis, represented as Kaplan-Meier survival plots was used to compare PFS in NSCLC patients with PD-L1 TPS ≥80% (red) and PD-L1 TPS 50-79% (blue).

Figure 3: Smoking history influences PFS in NSCLC patients prescribed pembrolizumab
Log-Rank analysis, represented as Kaplan-Meier survival plots was used to compare PFS in (A) smokers (former and current; red) and non-smokers (blue) and in (B) current smokers (red), former smokers (green), and non-smokers (blue).

Figure 4: Prior Radiotherapy influences PFS in NSCLC patients prescribed pembrolizumab
Log-Rank analysis, represented as Kaplan-Meier survival plots was used to compare PFS in NSCLC patients who received radiotherapy before or within two months of pembrolizumab (red) and those who did not receive radiotherapy in that time frame (blue).

Supplemental Material:

Supplementary Figure 1A: Further patient stratification by PD-L1 TPS does not influence OS in NSCLC patients
Log-Rank analysis, represented as Kaplan-Meier survival plots was used to compare OS in NSCLC patients with PD-L1 TPS ≥80% (red) and PD-L1 TPS 50-79% (blue).

Supplementary Figure 1B: Smoking history did not influence OS in NSCLC patients prescribed pembrolizumab
Log-Rank analysis, represented as Kaplan-Meier survival plots was used to compare OS in (A) smokers (former and current; red) and non-smokers (blue).

Supplementary Figure 1C: Prior Radiotherapy did not influence OS in NSCLC patients prescribed pembrolizumab
Log-Rank analysis, represented as Kaplan-Meier survival plots was used to compare OS in NSCLC patients who received radiotherapy before or within two months of pembrolizumab (red) and those who did not receive radiotherapy in that time frame (blue).

Supplementary Table 1: Summary of Patient demographics

Supplementary Table 2: Cox Regression analysis

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Mander *et al*, Figure 1

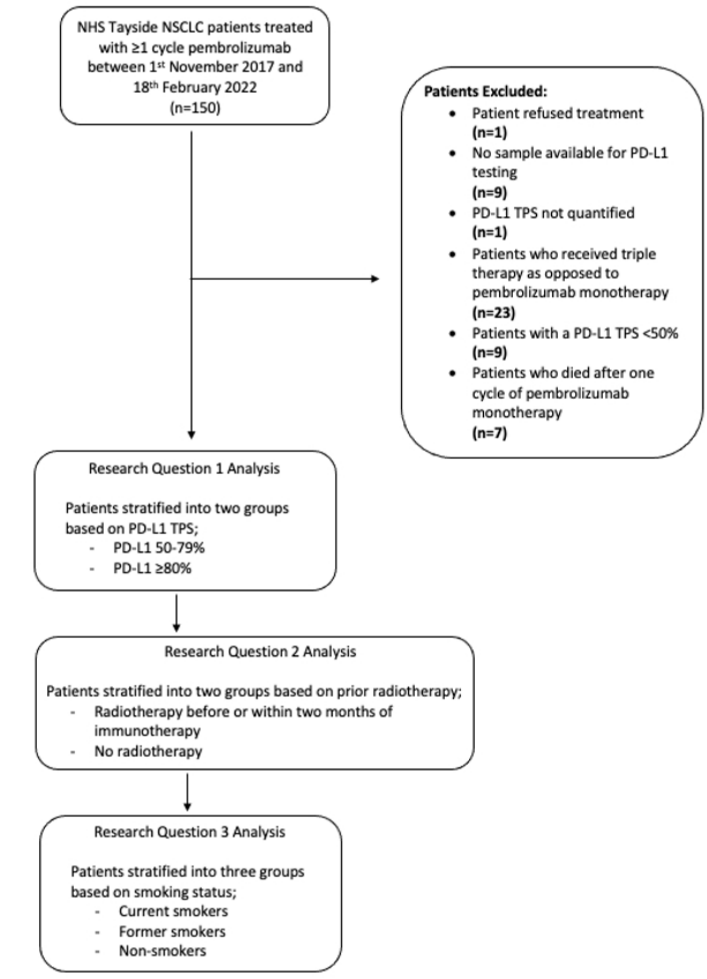


Figure 1: Patient Selection and Demographics

190x275mm (96 x 96 DPI)

Mander *et al*, Figure 2

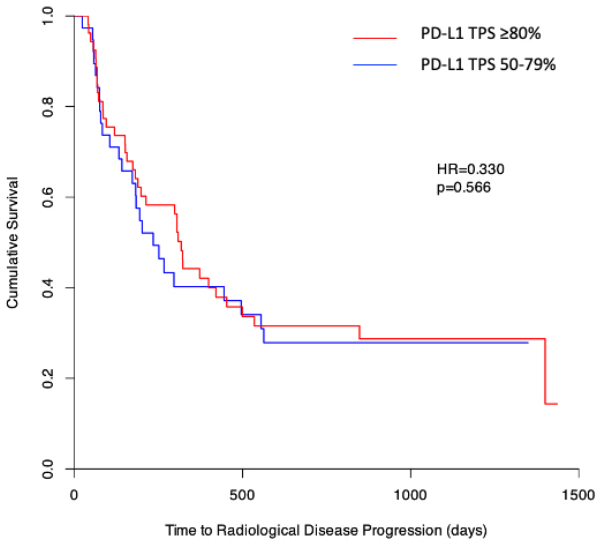


Figure 2: Further patient stratification by PD-L1 TPS does not influence PFS

190x275mm (96 x 96 DPI)

Mander *et al*, Figure 3

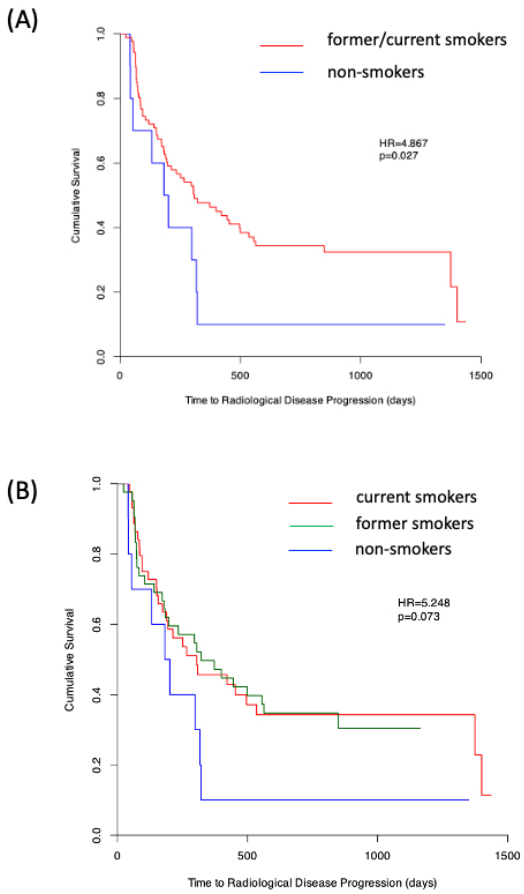


Figure 3: Smoking history influences PFS in NSCLC patients prescribed pembrolizumab

190x275mm (96 x 96 DPI)

Mander *et al*, Figure 4

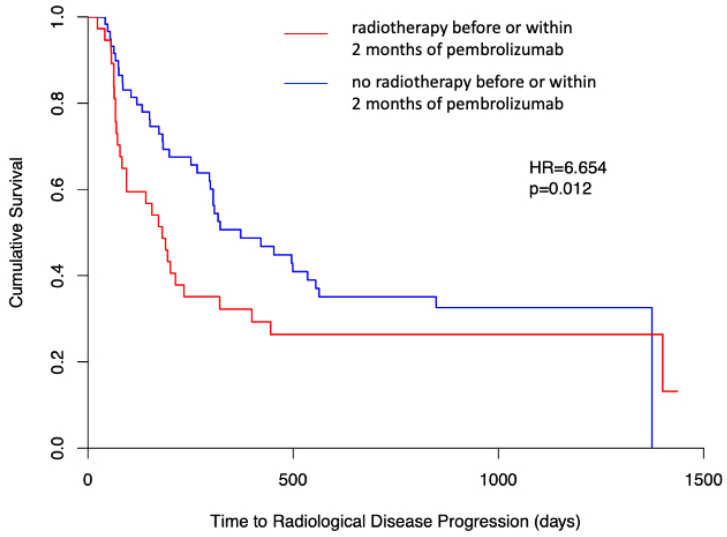


Figure 4: Prior Radiotherapy influences PFS in NSCLC patients prescribed pembrolizumab

190x275mm (96 x 96 DPI)

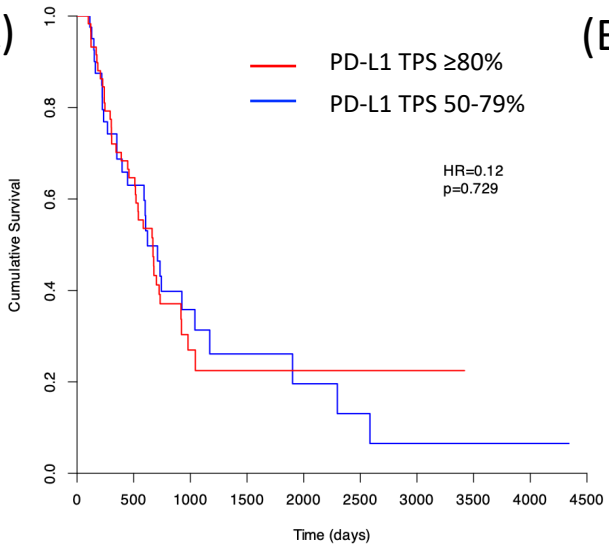
Mander *et al*, Supplementary Table 1
Summary of Patient demographics

Characteristics	PD-L1 TPS 50-79% (N = 40)	PD-L1 TPS ≥80% (N = 60)	p-value for pairwise comparison (Mann-Whitney test)
Age - yr (at diagnosis)			0.428
Median	67	68	
Range	47-81	40-91	
Sex			0.955
Male - no. (%)	18 (45.0%)	26 (43.3%)	
Female - no. (%)	22 (55.0%)	34 (56.7%)	
Performance status			0.353
0 - no. (%)	7 (17.5%)	8 (13.3%)	
0-1 - no. (%)	0 (0%)	3 (5%)	
1 - no. (%)	20 (50%)	35 (58.3%)	
1-2 - no. (%)	0 (0%)	3 (5%)	
2 - no. (%)	8 (20%)	10 (16.7%)	
2-3 - no. (%)	3 (7.5%)	0 (0%)	
3 - no. (%)	2 (5.0%)	1 (1.7%)	
Smoking status			0.306
Current - no. (%)	22 (55.0%)	23 (38.3%)	
Former - no. (%)	12 (30.0%)	31 (51.7%)	
Never - no. (%)	6 (15.0%)	6 (10.0%)	
Histology			0.280
Squamous cell carcinoma - no. (%)	12 (30.0%)	13 (21.7%)	
Adenocarcinoma- no. (%)	25 (62.5%)	41 (68.3%)	
Adenosquamous - no. (%)	1 (2.5%)	1 (1.7%)	
Not specified - no. (%)	2 (5.0%)	5 (8.3%)	
EGFR mutation status			0.692
Positive - no. (%)	1 (2.5%)	1 (1.7%)	
Negative - no. (%)	25 (62.5%)	43 (71.7%)	
Unknown - no. (%)	14 (35.0%)	16 (26.7%)	
ALK translocation status			
Positive - no. (%)	0 (0%)	0 (0%)	1.000
Negative - no. (%)	25 (62.5%)	43 (71.7%)	
Unknown - no. (%)	15 (37.5%)	17 (28.3%)	
ROS-1 rearrangement status			1.000
Positive - no. (%)	0 (0%)	0 (0%)	
Negative - no. (%)	10 (25.0%)	19 (31.7%)	
Unknown - no. (%)	30 (75.0%)	41 (68.3%)	
Pembrolizumab therapy			0.101
First line (%)	34 (85%)	54 (85%)	
Second line (%)	6 (15%)	6 (15%)	
Stage			0.422
I - no. (%)	1 (2.5%)	1 (1.7%)	
II - no. (%)	3 (7.5%)	2 (3.3%)	
III - no. (%)	10 (25.0%)	14 (23.3.%)	
IV - no. (%)	26 (65.0%)	43 (71.7%)	
Radiotherapy before or within two months of immunotherapy			0.944
Radical - no. (%)	4 (10.0%)	6 (10.0%)	0.968
Palliative - no. (%)	12 (30.0%)	17 (28.3%)	0.961
Thoracic - no. (%)	4 (10.0%)	10 (16.7%)	
Extra-thoracic - no. (%)	8 (20.0%)	7 (11.7%)	

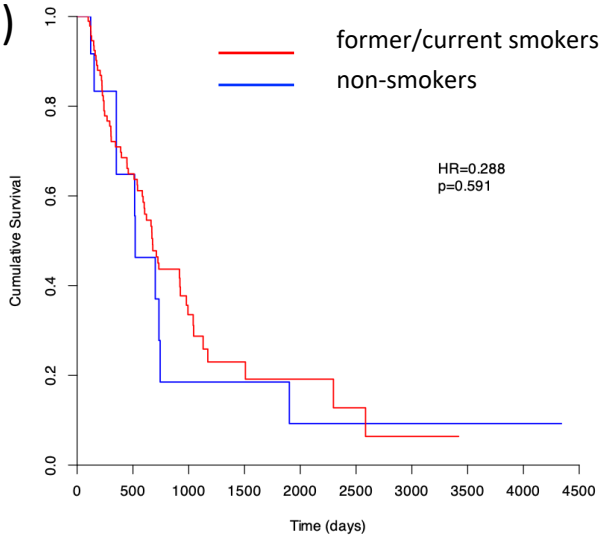
For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Mander *et al*, Supplementary Figure 1

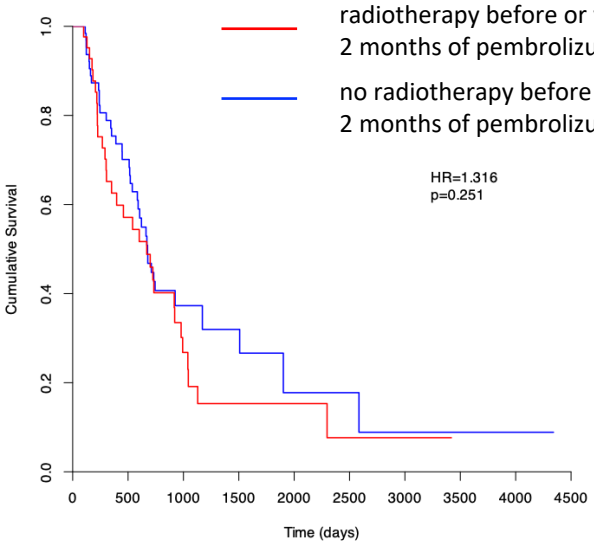
(A)



(B)



(C)



Mander *et al*, Supplementary Table 2
Cox regression analysis

Variable	Hazard Ratio (HR)	Standard Error (SE)	p-value
PD-L1≥80% (yes/no)	1.178	0.286	0.568
Smoking status (yes/no)	2.040	0.408	0.081
Performance Status (PS)			**0.009**
PS(1)	0.074	0.747	0.000
PS(2)	0.523	0.857	0.449
PS(3)	0.133	0.661	0.002
PS(4)	0.279	0.982	0.193
PS(5)	0.137	0.695	0.004
(PS(6)	0.073	1.191	0.028
Stage			0.126
Stage(1)	0.616	1.032	0.639
Stage(2)	0.310	0.657	0.075
Stage(3)	0.504	0.370	0.064
Histology			0.827
Histology(1)	1.028	1.041	0.979
Histology(2)	1.237	1.071	0.843
Radiotherapy before of within 2 months of starting immunotherapy (yes/no)	0.478	0.322	**0.022**