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Impact of Tumor Mutational Burden and Gene Alterations Associated with Radiation-Response on Outcomes of Post-Operative Radiation Therapy in Non-Small Cell Lung Cancer

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Abstract

Introduction: Post-operative radiation therapy (PORT) in resected NSCLC improves local-regional outcomes but recent randomized data do not support its unselected use. We assessed if tumor mutational burden (TMB) and mutations in genes associated with radiation sensitivity can select patients for PORT.

Methods: Patients with resected NSCLC treated with and without PORT who underwent tumor genomic profiling were examined. The incidence of local-regional failures (LRF) in patients with deleterious mutations in DNA damage response and repair (DDR) genes and genes associated

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with radiation-resistance (*KEAP1/NFE2L2/STK11/PIK3CA*) were investigated. Cox modeling and receiver operating characteristic curve (ROC) analysis assessed the relationship between TMB and local-regional control (LRC).

Results: Eighty-nine patients with NSCLC treated with PORT were analyzed with 2-year LRF rate of 19% (95% CI: 10–27%). Among PORT patients, those with mutations in radiation-resistance genes (n=16, 18%) had significantly more LRF than patient without (2-year LRF: 60% vs 11%, $p<0.001$). On multivariate analysis (MVA), radiation-resistance mutations associated with LRF after PORT (HR, 7.42, $p<0.001$). Patients with mutations identified in DDR genes (n=15, 17%) had significantly improved LRC ($p=0.048$) and no LRF events after PORT. On MVA, a higher TMB associated with improved LRC after PORT (HR, 0.86, $p=0.01$) and TMB was associated with PORT outcomes (area under ROC curve: 0.67–0.77). These genomic markers did not similarly associate with LRF in patients without PORT.

Conclusion: Our data suggests that patients with radiation-resistance gene alterations may derive minimal benefit from PORT, whereas patients with high-TMB and/or alterations in DDR genes may benefit from PORT and be suited for future precision-RT strategies. Prospective studies are necessary to validate these findings.

Keywords

post-operative radiation therapy; non-small cell lung cancer; radiation resistance; DNA damage repair; tumor mutational burden

Introduction:

Improved local-regional disease control has been found to translate to superior survival outcomes in patients with non-small cell lung cancers (1–3). Post-operative thoracic radiation therapy (PORT) after surgical resection has therefore been a standard adjuvant treatment offering in patients with adverse pathological features, namely mediastinal node positivity. However, recent prospective data from the LungART and PORT-C trials suggest that PORT should not be universally recommended, and that strategies to better identify and select patients who would most benefit from PORT are needed (4, 5).

Despite the conflicting results on the impact of PORT on disease-free survival, both the LungART and PORT-C trials found PORT to significantly improve local-regional outcomes supporting its role in the management of resected NSCLC (4, 5). There is now a greater understanding of how tumor genetics contribute to radiation response and of the radiation-induced toxicities of PORT (5, 6). Therefore, tumor genomics could potentially be used to select patients who would most benefit from PORT and allow for precision radiation that can improve the therapeutic ratio.

In patients with NSCLC, tumor mutations in *STK11, KEAP1, NFE2L2* and *PIK3CA* genes have been associated with radiation resistance, with treatment failures even after high-dose SBRT (7–9). On the other hand, mutations in DNA damage response and repair (DDR) genes have been associated with more favorable local-regional outcomes (10). Additionally, although there are limited data associating tumor mutational burden (TMB) with radiation response, multiple reports have found a higher TMB to associate with both DDR mutations

and tumor immunogenicity, suggesting its potential utility as a novel radiation-response biomarker (11–14).

We, therefore, assessed patients with resected NSCLC treated with PORT who underwent tumor genomic profiling to determine if tumor genomics and TMB could be used to identify patient subgroups who may and may not benefit from PORT and guide PORT dosing. We also explored a cohort of resected NSCLC patients who although were candidates, did not receive PORT to assess if these genomic biomarkers are uniquely predictive of PORT outcomes. We hypothesized that patients with tumor mutations in genes associated with radiation-resistance would have limited benefit from PORT, whereas patients with tumor mutations in DDR genes and high-TMB would have favorable local-regional outcomes and be best suited for future precision-RT approaches.

Methods:

PORT Patients and Treatment:

We retrospectively examined consecutive patients with NSCLC treated with curative intent surgery who received PORT between January 2017 through September 2019. Evaluated patients gave informed consent, and underwent, targeted next generation sequencing (MSK-IMPACT; Integrated Mutation Profiling of Actionable Cancer Targets) (15, 16). Next generation sequencing was performed on available tissue from the primary tumor or regional nodal metastases. This research was conducted in accordance with the US Common Rule, and this study was Institutional Review Board approved.

Standard pre-treatment evaluation included a physical examination, computed tomography (CT) scan of the chest, abdomen and pelvis and/or whole-body fluorine-18 fluorodeoxyglucose positron emission tomography (PET), and magnetic resonance imaging (MRI) of the head when appropriate. Indications for PORT included mediastinal node positivity or positive surgical margin status. The standard PORT radiation dose was 54Gy in 1.8Gy fractions but ranged from 50 – 60Gy and treatment was standardly delivered using intensity-modulated radiation therapy. Tumor genomic profiling data were not used in PORT clinical decision-making. Radiation treatment planning included a 4-dimensional CT simulation and PORT target volumes included the involved nodal stations, bronchial stump an ipsilateral hilum (17). Adjuvant and neoadjuvant platinum-based systemic therapy was as per standard of care. PORT standardly followed after the completion of platinum-based systemic therapy. Additionally, a minority of patients received either EGFR directed tyrosine kinase inhibitor (TKI) or immune checkpoint inhibitor (ICI) on investigational protocols. Imaging with chest CT was performed every 6 months, or more frequently as clinically warranted. All patients suspected of disease progression underwent PET/CT imaging, and whenever feasible, biopsy.

Tumor Genomic Profiling and PORT Patient Cohorts:

Two cohorts of patients treated with PORT were defined through tumor genomic profiling based upon the presence of deleterious mutations (1) patients with mutations in genes associated with radiation resistance and (2) patients with mutations in DDR genes with no

identified mutations in radiation resistance genes. Deleterious mutations included truncating, frame shift, splicing and fusion mutations predicted to impair protein function including as well as missense mutations predicted to be pathogenic based on OncoKB and/or ClinVar and literature review (18).

Investigated genes associated with radiation resistance included *STK11*, *KEAP1*, *NFE2L2* and *PIC3KA*, as these genes all have both pre-clinical and clinical data demonstrating them to associate with radiation resistance in patients with NSCLC (7–9, 19). A panel of 43 genes involving major DNA damage and response pathways were selected based upon prior investigation (10, 20–22). (Supplemental Table 1).

Exploratory No-PORT Cohort:

To determine whether associations that were observed within the PORT cohort were radiation-specific and distinct from those patients receiving surgery and systemic therapy alone, a cohort of consecutive patients with AJCC 8th edition stage III NSCLC with pathological involved mediastinal nodes also treated between January 2017 through September 2019 with curative surgery but who did not receive PORT were also reviewed. Patients gave informed consent, and underwent, targeted next generation sequencing as aforementioned. No-PORT patients were similarly examined based on presented of tumor mutations associated with radiation resistance and mutations in DDR genes.

Statistical Analysis:

Data on patient age, sex, stage, smoking history, histology, Eastern Cooperative Oncology Group (ECOG) performance status, surgery type, surgical margin status, involved mediastinal nodal stations, TMB in units of mutations per megabase (mt/Mb), systemic therapy, and radiation dose and technique were collected. Baseline characteristics between PORT patients with and without tumor mutations in genes associated with radiation resistance and in patients with and without tumor mutations identified only in DDR genes were compared using the chi-square test, Fisher's exact or the Wilcoxon test. We assessed for association between patient and tumor characteristics and local-regional failure using univariate and multivariate Cox proportional hazards modeling. Variables with $p < 0.05$ on univariable analysis were analyzed in multivariate analysis. TMB was evaluated as a continuous variable, and the number of involved mediastinal stations was assessed categorically as < 2 stations vs ≥ 2 stations.

Among PORT patients, overall survival was defined from the start of radiotherapy to disease-progression or death. Local-regional failure (LRF) and distant-metastasis free survival (DMFS) were defined from the start of radiotherapy to disease progression, with distant failure defined as metastatic disease progression per AJCC 8th edition staging. Among patients not treated with PORT, outcomes were defined from the date of surgery. Investigators were blinded to tumor mutation results when determining disease status. Patients were censored from analysis at time of their first progression event. Kaplan-Meier analysis was used to determine overall survival, cumulative incidence of LRF and DMFS. The log-rank test was used to compare overall survival, LRF and DMFS between patients with and without deleterious mutations in genes-associated with radiation resistance and

with and without deleterious mutations identified only in DDR genes. Additionally, receiver operator characteristic curve analysis was performed to assess the relationship between TMB and LRF among patients with PORT. Differences were described as statistically significant for p-values < 0.05. All statistical computations were performed using SPSS software Version 27 (IBM, Armonk, NY).

Results:

Characteristics of Patients Treated with PORT:

We identified 89 consecutive patients who received PORT and had tumor genomic profiling completed on their primary or regional disease. Most patients had stage IIIA or IIIB disease (n=81, 91%), adenocarcinoma histology (n=78, 88%) and underwent a lobectomy (n=79, 89%) with a negative-margin resection (n=78, 88%). In total, 89% (n=79) received platinum-based chemotherapy either as neoadjuvant (n=32) or adjuvant (n=46) therapy. Additionally, 14% (n=12) and 11% (n=10) of patients received ICI or TKI therapy, respectively (Table 1). The median TMB was 7 mt/Mb. The PORT prescription most prescribed was 54Gy in 1.8Gy fractions (n=76, 85%) and 80% (n=71) received IMRT. Median follow-up after PORT was 36 months (IQR: 27 – 43 months).

In total, 16 (18%) PORT patients had a deleterious mutation in a gene associated with radiation-resistance: *STK11* (n=11), *KEAP1* (n=4), *NFE2L2* (n=1) and *PIK3CA* (n=2). Patients with and without these radiation-resistance tumor mutations were similar in stage, surgical margins status, number of involved mediastinal stations and TMB, but patients without mutations were older in age (p=0.006). Table 2A.

In total, 15 (17%) PORT patients had a deleterious tumor mutation identified only in a DDR gene. Most common deleterious DDR mutations occurred in *ARID1A* (n=4), *ATM* (n=3), *POLE* (n=3), *PMS2* (n=2) and *TP53BP1* (n=2). Patients with and without these DDR mutations were mostly similar disease and treatment characteristics, but patients with mutations were found to have a significantly higher TMB (median 14 vs 6.1 mt/Mb, p=0.003). Table 2B. Among patients with deleterious tumor mutations in DDR genes, 6 received neoadjuvant chemotherapy, of whom all had >10% viable tumor remaining within the tumor bed after chemotherapy. Tumor genomic profiling results are shown in Supplemental Table 2.

Overall Disease and Treatment Outcomes Among PORT Patients:

Across all patients treated with PORT, the 2 and 3-year incidence of LRF was 19% (95% CI: 10 – 27%) and 30% (13 – 42%), respectively. In total, 21 patients developed LRF and all but one patient had of component of an in-field failure seen on imaging. In total, 7 patients had local, in-field failure within the lung parenchyma and 14 patients had regional, in-field failure within the thoracic nodes. The 2- and 3-year DMFS estimates were 68% (58 – 78%) and 60% (49 – 71%). The median OS was not reached, the 2 and 3-year OS estimates were 78% (70 – 87%) and 71% (61 – 81%), respectively. In total, 43% (n=38) of patients had recurrent disease, and most patients (89% of recurrences, n=34) had a component of distant metastatic disease at first relapse.

Mutations in Radiosensitivity Genes Predict Local-Regional Outcomes with PORT.

Patients with deleterious tumor mutations in genes associated with radiation resistance had a significantly higher rate of LRF after PORT compared to patients without deleterious mutations in radiation resistance genes (p<0.001). The 2-year cumulative incidence of LRF in patients with vs without deleterious mutations was 60% (33 – 87) vs 11% (3 – 18%). (Figure 1A).

Patients with deleterious tumor mutations identified only in DDR genes had a significantly lower incidence of LRF (p=0.048) after PORT compared to patients without deleterious DDR mutations. There were no LRF events among these patients with mutations vs a 2-year incidence of LRF of 22% (12 – 32%) in patients without mutations identified in DDR genes. (Figure 1B).

TMB and Mutations in Radiation-Resistance Genes Predict Benefit of PORT

On univariate analysis neither age, sex, ECOG status, margin status, number of involved mediastinal stations nor receipt of ICI or TKI associated with LRF after PORT. On univariate analysis, a higher-TMB (p = 0.04) associated with improved LRC, and the presence of a deleterious radiation resistance mutation (p < 0.001) associated with increased LRF after PORT. On multivariate analysis, higher-TMB [hazards ratio (HR), 0.86, 95% CI, 0.77 – 0.97, p = 0.01] independently associated with improved LRC, and deleterious radiation resistance mutations (HR, 7.42, 95% CI, 2.83 – 19.44, p < 0.001) independently associated with increased LRF after PORT (Table 3).

Across all PORT patients, receiver operating characteristic curve analysis of TMB and local regional control found an area under the curve of 0.67 (0.54–0.8) (Figure 2A). When excluding patients with tumor mutations in radiation resistance genes, ROC analysis of TMB and LRF found an area under the curve of 0.77 (0.62–0.92) with a high-TMB (≥ 10 mt/Mb) having a 92% sensitivity for predicting LRC after PORT (Figure 2B).

Distant Control and Overall Survival After PORT in Radiation Resistant and Sensitive Cohorts:

Patients with radiation resistance mutations had significantly lower DMFS and overall survival versus patients without: two-year DMFS and OS rates of 25% (5 – 45%) vs 77% (68 – 87%) and 53% (27 – 79%) vs 83% (75 – 92%) (p<0.001, for both comparisons). (Supplemental Figure 1). Among patients with mutations only in DDR genes, DMFS and overall survival were not significantly different between patients with and without mutations (p=0.944 and p=0.25, respectively) (Supplemental Figure 2). However, compared to patients with radiation resistance mutations, patient with DDR mutations had significantly higher DMFS, with 2-year DMFS rates of 67% (33 – 91%) vs 25% (5 – 45%) (p=0.019) (Supplemental Figure 3).

Genomic Predictors for PORT Outcomes Do Not Associate with LRC in No-PORT Cohort

In total, 19 patients with stage III NSCLC with mediastinal nodal involvement who did not receive PORT were examined (Supplemental Table 3). Among these patients, 4 (21%) had a deleterious mutation in genes associated with radiation-resistance and 4 (21%)

had a deleterious mutation identified only in a DDR Gene (Supplemental Table 4). The 2-year incidence of LRF was 57% (95% CI: 30–86%) with no significant difference in local-regional failure among patients with and without mutations in genes associated with radiation-resistance ($p = 0.99$) or between patients with and without mutations identified only in DDR genes ($p = 0.322$) (Supplemental Figure 4). On univariate variate analysis, radiation-resistance or DDR gene mutational status did not predict for local-regional failure, but a higher-TMB was associated with increased LRF (HR, 1.28, 95% CI, 1.05 – 1.55, $p = 0.01$) (Supplemental Table 5).

Discussion:

Recently published randomized trials evaluating PORT in patients with resected NSCLC have called out the need to better identify patients who may most benefit from PORT (4, 5). Although adjuvant therapy in resected NSCLC is evolving, local-regional failures are the predominant site of relapse even in patients treated with adjuvant ICI on recent trials (23). In this study, we assessed if tumor genomic profiling can be used to select patients for PORT. We found patients with mutations in tumor genes associated with radiation resistance to have high-rates of local-regional failure after PORT, suggesting a minimal benefit from PORT. However, in patients with mutations in DDR genes, local-regional failure was exceedingly low, suggesting that these patients not only benefit from PORT, but that a lower-dose precision-RT based approach may be warranted. Additionally, to our knowledge, this report is among the first to associate TMB with radiotherapy outcomes. A higher TMB has been associated with tumor DDR mutations and tumor immunogenicity (11, 12), and our data suggest that TMB may also be a novel biomarker for radiation-response. Given that we did not find these same genomic markers to associate with local-regional outcomes in patients without PORT, these markers potentially could be uniquely predictive of PORT outcomes and prospective studies are warranted to validate these findings.

We found patients with identified deleterious mutations in either *KEAP1*, *NFE2L*, *STK11* or *PIK3CA* to have a two-year LRF rate of approximately 60%. This high LRF rate suggests a limited benefit of PORT in this radiation-resistant subgroup as the 3-year LRF rate in the no-radiation arms of the PORT-C and LungART trials was approximately 45% (4, 5). The *KEAP1*/*NFE2L2* pathway plays a role in regulating cellular stress, and mutations in these genes can lead to NFE2L2 overexpression thereby protecting cancer cells from the effects of radiation (8, 24, 25). Prior studies have not found mutations in the *KEAP1*/*NFE2L2* pathway to predict for increased LRF in surgically treated patients without radiation or chemotherapy (8), suggesting the utility of *KEAP1*/*NFE2L2* in predicting radiotherapy local-regional outcomes. Mutations in STK11 have also been found to promote resistance to radiation potentially through engaging the KEAP1/NFE2L2 pathway (7). Additionally, mutations in PIK3CA have been associated with radiation resistance both in pre-clinical models and in patients with NSCLC treated with radiation (19, 26). Approximately 20% of our patient population had an identified radiation-resistance mutation. This suggests that this sizeable cohort of patients with inherent radiation-resistance could have blunted the benefit of PORT across the unselected patient populations in the PORT-C and LungART trials. While our data requires further validation, standard PORT among this patient subgroup may expose patients to RT-associated toxicity without significant clinical benefit.

Patients with tumor mutations identified only in DDR genes were found to be at very-low risk for LRF, with no LRF events in this patient subgroup. This finding cannot be fully explained by a favorable response to chemotherapy as all patients in the DDR mutant cohort who received neoadjuvant chemotherapy had viable tumor at time of surgical resection. Therefore, suggesting that PORT did indeed contribute to their favorable local-regional outcomes. Across the large panel of DDR genes selected, deleterious mutations in *ATM*, *POLE*, *PMS2*, *TP53BP1* or *ARID1A* were most identified. Importantly, while mutations in *ATM* and *TP53BP1* are central mediators in double-strand DNA break repair and are associated with clinical radiation sensitivity (27, 28), the role *POLE*, *ARID1A* and *PMS2* play in repair from DNA damage from radiotherapy is less clear (21, 29–31). Supporting our findings however is a similar analysis that assessed a panel of DDR genes from multiple DDR pathways and found NSCLC tumors with deleterious DDR mutations to have significantly improved local-regional control when treated with definitive chemoradiation (10). Given their exceedingly low rate of LRF with standard PORT, our data supports investigating a lower dose precision-PORT approach among these patients to improve the therapeutic ratio.

Most intriguing, we found a higher-TMB to predict for improved local-regional control after PORT on multivariate analysis. Additionally, TMB was identified as an acceptable-to-good tool on ROC analysis with high-sensitivity for identifying the benefit of PORT. A high-TMB has been found to predict for response to immunotherapy and pembrolizumab is FDA approved for the treatment of solid tumors based on high-TMB (32–34). However, TMB is not a prognostic biomarker as it has not been found to predict for outcomes in patients who have not received immunotherapy (33). Given that only 14% of patients in our study were treated with adjuvant ICI, our data imply that the association between TMB and local-regional outcomes is a reflection on radiation therapy. Furthermore, among our cohort of patients not treated with PORT, a higher-TMB was associated with *higher* local-regional failure. This finding is supported by a recent study that found higher-TMB to associate with aggressive clinicopathologic features that predict for local-regional recurrence (35, 36). All together, these data suggest that TMB is a radiation sensitivity biomarker that warrants investigation.

Multiple lines of evidence provide rationale to support our finding that a high-TMB can predict for radiation sensitivity. First, studies have found a higher TMB to correlate with DDR mutations and for the majority of NSCLC patients harboring DDR mutations to have a high-TMB (12–14). Given that DDR genes play a role in radiation repair, mutations in these genes, as our data also suggests, can lead to radiation sensitivity. Second, a higher TMB has been associated with tumor immunogenicity, as high-TMB tumors have more neoantigens that could be involved in antitumor immunity (11). Data have found radiation sensitivity to also be partly dependent on the anti-tumor immune response, therefore providing further biological rationale to support our findings (37, 38). Although validation of our work is necessary in other NSCLC patient populations with limited ICI exposure, TMB could represent a tool to select patients for precision-RT PORT approaches.

This work is limited by its retrospective nature and of its inclusion of a single cancer center. However, patients in this cohort had substantial follow-up and represent a modern cohort

during which time tumor genomic profiling was routinely performed on primary tumor specimens. Given that the determination of the pathogenicity of a mutation is dependent on available data, our work is further constrained by incomplete data on certain tumor mutations. Additionally, given the rare frequency of mutations in individual DDR genes, we used a previously established panel of genes for analysis, however this work is limited by its sample size and larger cohorts will be necessary to identify outcomes from mutations in individual repair genes. Another limitation of this analysis is our inability to simultaneously control for TMB and DDR mutations on MVA, due to the lack of events in the DDR mutant group. Additionally, although our no-PORT cohort was limited in size given that these patients deviated from an institutional standard of care, the findings from this cohort are consistent with the published literature. There were imbalances in characteristics between PORT patients with and without pathogenic radiation resistance mutations, with patients with mutations being younger in age, that could have introduced bias. Additionally, multiple hypotheses were tested which could have inflated type I error, however our statistical methods are consistent with the exiting literature in this space (7, 24, 39, 40).

Local-regional failures in resected NSCLC patients represent a predominant site of relapse. PORT has been found to significantly improve local-regional outcomes but strategies to select patients for PORT have been limited. We found that tumor genomic profiling can potentially identify patients with inherent radiation -resistance for whom standard PORT may have limited clinical benefit and that there may be a cohort of patients with DDR mutations and high TMB for whom a precision-RT based PORT treatment warrants further prospective investigation. Although further work validating these findings are required, strategies that involve tumor genomic may allow for optimal patient selection for PORT.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Research data are not available at this time, deidentified data elements can be made available upon reasonable request from the corresponding author.

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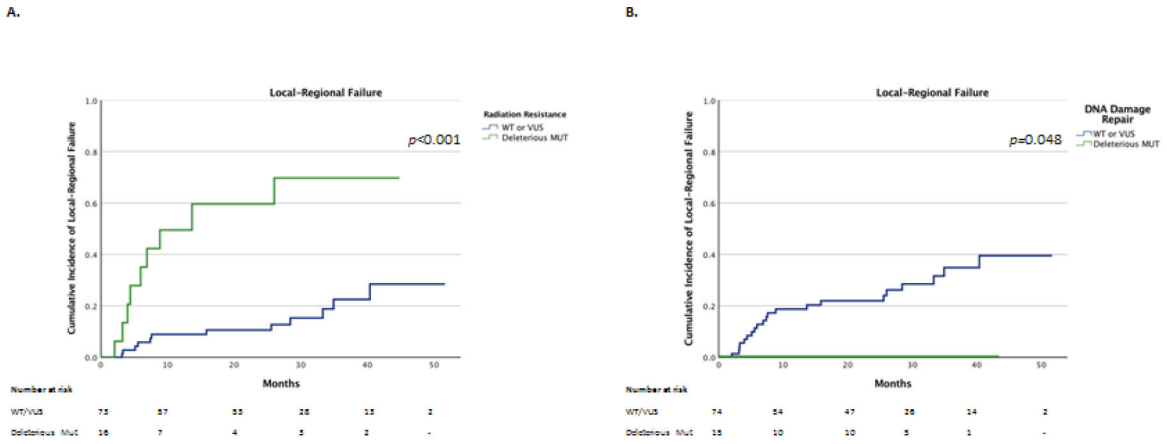

Figure 1.

Comparison of local-regional failures between PORT patients with and without deleterious tumor mutations in genes associated with radiation resistance (A) and with and without identified deleterious tumor mutations only in DDR genes (B).

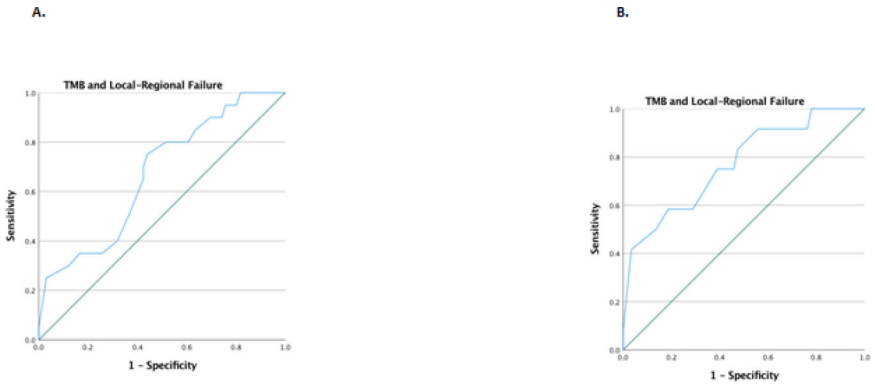

Figure 2.

Receiver operating characteristic curve analysis of TMB and local regional control among all PORT patients (A) and PORT patients without deleterious tumor mutations in genes associated with radiation resistance (B)

Table 1.

Patient and Treatment Characteristics

Characteristic	All Patients (n = 89) No. of Patients (%)
Median age, range	68 (46 – 83)
Smoking History	
Never	19 (21)
Former	66 (74)
Current	4 (5)
Sex at Birth	
Female	57 (64)
Male	32 (36)
Performance Status	
ECOG 0	54 (61)
ECOG 1	35 (39)
Histology	
Adenocarcinoma	78 (88)
Squamous Cell	6 (7)
Other	5 (5)
Tumor Mutational Burden	
Median, IQR (mt/Mb)	7 (3.5 – 11.63)
AJCC 8th Overall Stage	
I	1 (1)
IIB	7 (8)
IIIA	67 (75)
IIIB	14 (16)
Surgery Type	
Wedge / Segmentectomy	8 (9)
Lobectomy	79 (89)
Pneumonectomy	2 (2)
Margin Status	
Negative	78 (88)
Positive	11 (12)
Involved Mediastinal Nodal Stations	
0	10 (11)
1	53 (60)
2	23 (26)
3	3 (3)

Characteristic	All Patients (n = 89) No. of Patients (%)
Chemotherapy	
Yes	79 (89)
Neoadjuvant	32 (46)
Adjuvant	46 (52)
Neo/Adjuvant ICI	
Yes	12 (14)
Adjuvant TKI	
Yes	10 (11)
Radiation Dose	
Median, range (Gy)	54 (50 – 60)
Radiation Technique	
3D-CRT	18 (20)
IMRT	71 (80)

Table 2A.

Patient and Treatment Characteristics

	No. of Patients (%)		
Characteristic	**Radiation Resistance WT or VUS (n = 73)**	**Radiation Resistance mt (n = 16)**	***p – value***
Age			0.006
Median, Range (years)	68 (52 – 83)	64 (46 – 79)	
Ever Smoker			0.104
Yes	55 (75)	15 (94)	
Sex			0.665
Female	46 (63)	11 (69)	
Male	27 (37)	5 (31)	
Performance Status			0.689
ECOG 0	45 (62)	9 (56)	
ECOG 1	28 (38)	7 (44)	
Histology			0.391
Adenocarcinoma	65 (89)	13 (81)	
Other	8 (11)	3 (19)	
Tumor Mutational Burden			0.163
Median, IQR (mt/Mb)	6.1 (2.6–10.5)	10.5 (6.1–15.8)	
AJCC 8th Overall Stage			0.672
< III	7 (10)	1 (6)	
IIIA or IIIB	66 (90)	15 (94)	
Margin Status			0.985
Negative	64 (88)	14 (88)	
Positive	9 (12)	2 (12)	
Involved Mediastinal Nodal Stations			0.158
<2	54 (74)	9 (56)	
≥2	19 (26)	7 (44)	
Received Chemotherapy			0.116
Yes	63 (86)	16 (100)	
Received ICI			0.350
Yes	11 (15)	1 (6)	
Received TKI			0.113
Yes	10 (14)	0	
Radiation Dose			0.616

	No. of Patients (%)		
Characteristic	**Radiation Resistance WT or VUS (n = 73)**	**Radiation Resistance mt (n = 16)**	***p – value***
Median, range (Gy)	54 (50 – 60)	54 (50.4 – 60)	
Radiation Technique			
3D-CRT	15 (20)	3 (19)	0.871
IMRT	58 (80)	13 (81)	

Table 2B.

Patient and Treatment Characteristics

	No. of Patients (%)		
Characteristic	**DDR WT or VUS (n = 74)**	**DDR mt (n = 15)**	***p – value***
Age			0.951
Median, Range (years)	68 (46 – 83)	69 (52 – 80)	
Ever Smoker			0.406
Yes	57 (77)	13 (87)	
Sex			0.720
Female	48 (65)	9 (60)	
Male	26 (35)	6 (40)	
Performance Status			0.523
ECOG 0	46 (62)	8 (53)	
ECOG 1	28 (38)	7 (46)	
Histology			0.9
Adenocarcinoma	65 (88)	13 (87)	
Other	9 (12)	2 (13)	
Tumor Mutational Burden			0.003
Median, IQR (mt/Mb)	6.1 (3–10.5)	14 (7.9–19.4)	
AJCC 8th Overall Stage			0.182
< III	8 (11)	0	
IIIA or IIIB	66 (89)	15 (100)	
Margin Status			0.9
Negative	65 (88)	13 (87)	
Positive	9 (12)	2 (13)	
Involved Mediastinal Nodal Stations			0.314
<2	54 (73)	9 (60)	
≥2	20 (27)	6 (40)	
Received Chemotherapy			0.778
Yes	66 (89)	13 (87)	
Received ICI			0.397
Yes	11 (15)	1 (7)	
Received TKI			0.587
Yes	9 (12)	1 (7)	
Radiation Dose			0.616

	No. of Patients (%)		
Characteristic	**DDR WT or VUS (n = 74)**	**DDR mt (n = 15)**	***p – value***
Median, range (Gy)	54 (50 – 60)	54 (50 – 60)	
Radiation Technique			
3D-CRT	16 (22)	2 (13)	0.862
IMRT	58 (78)	13 (87)	

Table 3:

Predictors for Local-Regional Failure After PORT

	Univariate		Multivariate	
	HR (95% CI)	***p* - value**	**HR (95% CI)**	***p* - value**
Age	1.01 (0.95 – 1.06)	0.84		
Sex	1.67 (0.7 – 3.96)	0.25		
ECOG 0	1.47 (0.62 – 3.46)	0.38		
Margin Status	1.27 (0.37 – 4.29)	0.71		
Involved N2 Stations	2.05 (0.84 – 4.97)	0.11		
Receipt of ICI	0.53 (0.12 – 2.29)	0.39		
Receipt of TKI	0.32 (0.04 – 2.39)	0.27		
TMB	0.91 (0.83–0.99)	0.04	0.86 (0.77 – 0.97)	0.01
Radiation Resistance mut	5.41 (2.26–12.94)	<0.001	7.42 (2.83 – 19.44)	<0.001

Age assessed as continuous variable. Sex (male vs female [ref]); ECOG 0 [ref] vs ECOG 1. Margin status (positive vs negative [ref]). Involved N2 stations (2 stations [ref] vs ≥2 stations). Receipt of ICI (yes [ref] vs no). Receipt of TKI (yes [ref] vs no). TMB assessed as continuous variable. Radiation Resistance mut (yes vs no [ref]).