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Prognostic value and molecular correlates of a CT image-based quantitative pleural contact index in early stage NSCLC

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Abstract

Purpose—To evaluate the prognostic value and molecular basis of a CT-derived pleural contact index (PCI) in early stage non-small cell lung cancer (NSCLC)

Experimental design—We retrospectively analysed seven NSCLC cohorts. A quantitative PCI was defined on CT as the length of tumour-pleura interface normalised by tumour diameter. We evaluated the prognostic value of PCI in a discovery cohort (n=117) and tested in an external cohort (n=88) of stage I NSCLC. Additionally, we identified the molecular correlates and built a gene expression-based surrogate of PCI using another cohort of 89 patients. To further evaluate the

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Ethical approval:

Institutional Review Board approval was obtained.

Methodology:

- retrospective
- cross sectional study
- multicentre study

prognostic relevance, we used four datasets totalling 775 stage I patients with publically available gene expression data and linked survival information.

Results—At a cutoff of 0.8, PCI stratified patients for overall survival in both imaging cohorts (log-rank $P=0.030$, 0.0076). Extracellular matrix (ECM) remodelling was enriched among genes associated with PCI ($P=0.0003$). The genomic surrogate of PCI remained an independent predictor of overall survival in the gene expression cohorts (hazard ratio: 1.46, $P=0.0007$) adjusting for age, gender, and tumour stage.

Conclusions—CT-derived pleural contact index is associated with ECM remodelling and may serve as a noninvasive prognostic marker in early stage NSCLC.

Keywords

Pleural contact; Lung cancer; Prognosis; Imaging biomarker; Radiogenomics

Introduction

Lung cancer is the leading cause of cancer deaths in the United States, a majority (~85%) of which is non-small cell lung cancer (NSCLC)[1]. With recently approved reimbursement by the Centers for Medicare & Medicaid Services (CMS) for annual CT screening, the prevalence of early stage NSCLC is expected to increase[2]. Despite potentially curative treatment with surgery or stereotactic ablative radiotherapy (SABR), a significant portion (20–30%) of patients with early stage NSCLC will have a distant metastasis and may eventually die of their disease[3; 4]. There is a critical need to identify patients who are at high risk of relapse and thus require more extensive follow up or additional treatment[5].

Invasion and metastasis are hallmarks of cancer that transform a locally growing tumour into a systemic and lethal disease[6; 7]. Remodelling of extracellular matrix (ECM) plays an important role in cancer invasion[8; 9]. Although tightly controlled during normal organ development, ECM is commonly dysregulated in cancer, which disrupts tissue polarity and architecture and promotes invasion[10–13]. In order to migrate and invade into adjacent tissue, cancer cells have to overcome the physical barrier for cell escape. One key strategy is by overexpressing enzymes such as matrix metalloproteinases (MMP), which leads to breakdown and degradation of ECM and generates de novo space required for invasion[7]. Cancer cells that have evolved this capability have a selective advantage because they can readily enter and exit the basement membrane and metastasise to distant sites[8].

Lung tumours frequently invade surrounding tissue such as the pleura[14]. Indeed, pathological evidence of pleural invasion is an integral component in the tumour node metastasis (TNM) staging of lung cancer[15] where it determines if a tumour will move from T1 to T2. Pleural invasion has been shown to correlate with a poor prognosis in resected NSCLC[14; 16–18]. In recent meta-analysis of 22 studies[18], visceral pleural invasion was shown to be associated with poorer overall survival and a higher risk of recurrence in stage I patients, and was independent from tumour size and histology type. Previous efforts to search for noninvasive imaging predictors of pathological pleural invasion have led to mixed, ambiguous results[19; 20], as simple attachment to pleura does not

necessarily imply invasion. A quantitative measure of the exact extent of pleural contact with the tumour will provide more granular information and may be more accurate in determining the likelihood of pathological invasion and risk of recurrence and death.

In this work, we quantified the tumour-pleura contact length relative to tumour size at CT as a noninvasive image surrogate of pleural invasion. We hypothesised that the image finding is associated with ECM remodelling given its important role in cancer invasion. By integrating imaging with genomic data, we sought to evaluate the prognostic significance of the imaging marker and its radiogenomic signature in multiple independent cohorts of early stage NSCLC.

Material & Methods

Study design and patient cohorts

In this institutional review board approved study, we retrospectively analysed a total of 7 independent cohorts in order to develop and validate a CT image marker for predicting prognosis of early stage NSCLC patients. The entire study design is illustrated in Figure 1. Detailed description of the patient cohorts is provided below.

For imaging biomarker discovery, we retrospectively examined the records of patients who had biopsy-confirmed stage I primary NSCLC and treated with stereotactic ablative radiation therapy (SABR) at our institution from 2005 to 2013 ($n = 197$). Sixty-two patients were excluded due to receipt of previous treatment for NSCLC or additional systemic treatment before a diagnosis of metastatic failure, or a diagnosis of new primary NSCLC after SABR. Among the remaining patients, 117 had pre-treatment CT images and clinical outcomes available, which were retrospectively collected for this study.

To validate the proposed imaging marker, we used a NSCLC cohort with pre-treatment CT images and clinical outcomes publically available from the cancer imaging archive (TCIA) [21]. The original cohort consisted of 422 patients with inoperable, histologically or cytologically confirmed stage I to IIIB NSCLC who were treated with radiation therapy[22]. For our study, we only included patients with stage I NSCLC ($n = 93$), but excluded cases with incorrect staging information ($n = 2$) and tumours located inside mediastinum ($n = 3$), resulting in a cohort of 88 patients used for imaging marker validation. Table 1 shows the clinical and treatment information of both imaging discovery and validation cohorts.

In order to identify the biological underpinnings of the CT image marker, we analysed another lung cancer cohort[22] comprising 89 patients for whom both CT images and gene expression data were publically available from TCIA[23] and the gene expression omnibus (GEO), series number: GSE58661. This genomic discovery cohort was used to identify the molecular correlates as well as to build a gene expression-based surrogate of the CT image marker.

To further validate the genomic surrogate of the prognostic image marker, we searched for publically available gene expression data with linked survival information in online resources including PRECOG[24] and PROGgene[25]. We identified four largest NSCLC

datasets in terms of patient number and collected the normalised gene expression data from GEO (series number: GSE68465, GSE30219, GSE31210, GSE37745). For each cohort, we only retained the subset of stage I patients, with n=276, 201, 168, 130 patients for cohorts listed above, respectively. In addition, we applied standardised separators to remove the batch effect[26] and constructed a meta-cohort (n=775) by combining the four individual cohorts together.

Quantifying pleural contact on CT image and its reproducibility

Pleural contact index (PCI) was defined as the ratio between the total length of the tumour-pleural interface and the maximum tumour diameter on CT images similar to a previous study[27]. PCI was computed for each tumour in the imaging discovery and validation cohorts, and genomic discovery cohort (see Supplementary Information and Supplementary Figure 1). The reproducibility of PCI was evaluated by the intra-class correlation coefficient[28] and Bland-Altman analysis[29] based on tumour contours independently drawn by two radiation oncologists in the imaging discovery cohort. All image processing and analysis were performed using in-house software developed in MATLAB 2016a (MathWorks, Natick, Mass).

Assessing prognostic value and identifying molecular correlates of PCI

We validated the prognostic value of PCI in two phases. In the first phase, we performed direct validation of PCI on both internal and external imaging cohorts. We identified an optimal cutoff value for the PCI based on the discovery cohort and accordingly defined a dichotomous risk factor for PCI. The same cutoff value was applied to dichotomise PCI and evaluate the survival stratification on the external validation cohort. Information about overall survival (OS) was available for both imaging cohorts and was used as the primary endpoint. Additionally, information about distant metastasis free survival (DMFS) and local regional failure (LRF) was available for the imaging discovery cohort and was used as secondary endpoints. Further, we examined whether PCI provided complementary prognostic value to other imaging, clinical, and treatment factors including pleural contact length, presence of pleural attachment, tumour size, location (central versus peripheral), histology, age, gender, and biologically effective dose (BED). Central tumour location was defined as within 2 cm of the proximal bronchial tree, heart, great vessels, trachea, or other mediastinal structures[30].

In the second phase of validation, we built a gene-expression based classification model of the imaging phenotype (dichotomous PCI defined above) using the genomic discovery cohort. Specifically, we calculated the Spearman correlation coefficient between the PCI and expression of each gene. Expression of the top 50 genes were used as the input to train a nearest shrunken centroid classifier[31] to separate samples into low- versus high-PCI groups according to the optimal cutoff determined in the imaging discovery cohort (see Supplementary Information for details). Finally, we applied this radiogenomic classifier to stratify patients and evaluate their prognostic potential on four independent genomic validation cohorts and the combined meta-cohort.

In order to reveal the biological underpinnings of the imaging-derived PCI, we performed radiogenomic association analysis on the NSCLC genomic discovery cohort. We kept genes significantly correlated with PCI according to Spearman's rank test, with which we performed enrichment analysis using the function annotation tool of Database for Annotation, Visualization and Integrated Discovery (DAVID)[32]. Additionally, we performed unsupervised hierarchical clustering to determine whether there were subgroups in the cohort with distinct gene expression patterns associated with the PCI.

Statistical analysis

Estimates for probabilities of OS, DMFS, and LRF were computed using Kaplan-Meier analysis. An optimal cut-off value of PCI to separate high versus low-risk groups was obtained by maximising the hazard ratio of overall survival. The survival stratification of the two risk groups was assessed by the log-rank test. Univariate and multivariate analyses were performed using a Cox proportional hazard regression model. Prognostic accuracy of PCI was measured by Harrell's concordance index[33]. To adjust for multiple statistical testing, the Benjamini-Hochberg method[34] was used to control the false discovery rate (FDR) in the gene set enrichment analysis. All statistical analysis was conducted with R software package (Version 3.3.0).

Results

Prognostic value of PCI in two imaging cohorts

PCI showed a high degree of reproducibility against variations in tumour contours, with an intra-class correlation of 86%. The Bland-Altman analysis showed that mean differences of PCIs of two radiation oncologists were mainly bounded within 95% confidence intervals (Supplementary Figure 2). The optimal cut-off value for PCI was determined to be 0.8 for separating patients at high versus low risk for OS in the imaging discovery cohort. Kaplan-Meier analysis showed that PCI significantly stratified patients into two groups with different OS in the discovery cohort (Figure 2A), with a concordance index of 0.598 and hazard ratio of 2.388 (log-rank test $P = 0.0076$). In addition, PCI stratified patients into groups regarding distant metastasis (Figure 2B), with a concordance index of 0.668 and hazard ratio of 3.629 (log-rank test $P = 0.0060$), but was not associated local regional failure in the imaging discovery cohort. The prognostic value of PCI was confirmed in the imaging validation cohort (Figure 2C), with a concordance index of 0.589 and hazard ratio of 1.928 (log-rank test $P = 0.0304$) for OS. Among the subgroup of patients with pleural attachment (*i.e.*, $PCI > 0$), a higher PCI was significantly associated with worse OS in the imaging discovery cohort (log-rank test $P = 0.0234$). Due to a relatively small number of patients in the imaging validation cohort, there was a trend toward statistical significance in the association between PCI and OS (log-rank test $P = 0.1409$).

Complementary value of PCI to tumour size and other prognostic factors

On multivariate analysis including clinical, treatment, and imaging variables, PCI and age were independent predictors of OS in the imaging discovery cohort (Table 2). In the imaging validation cohort, while PCI was significantly associated with OS on univariate analysis, tumour volume was the only significant predictor of OS on multivariate analysis. With

respect to distant metastasis, several factors including PCI, tumour diameter, and tumour location were independent predictors on multivariate analysis. There were no statistically significant predictors of local regional failure in the imaging discovery cohort.

To further assess whether PCI provided additional prognostic value to tumour size, we performed subgroup analysis for patients with tumours <3 cm versus those >3 cm, *i.e.*, clinical stage IA versus IB. In the imaging discovery cohort, a higher PCI was significantly associated with worse OS within stage IA subgroup (log-rank test $P = 0.0306$). There was a similar trend in other subgroups for both cohorts although it did not reach statistical significance due to a limited number of patients (Figure 5). When assessed in the combined imaging discovery and validation cohorts, PCI significantly stratified patients for OS within both stage IA and IB subgroups (log-rank test $P = 0.0486$ and 0.0223 respectively) as shown in Figure 2D–E.

Molecular correlates of PCI

On the genomic discovery cohort, we identified 1919 genes that are significantly correlated with PCI (Spearman test $P < 0.05$). The top 10 gene ontologies identified by the functional enrichment analysis of DAVID using these genes were shown in Supplementary Table 1. Of note, biological processes related to extracellular matrix organisation (FDR = 0.005) and collagen catabolic process (FDR = 0.033) were enriched. Interestingly, tumours with higher PCI tended to have higher expression of MMP1 ($P = 0.014$), a key ECM degradation enzyme. Further, we performed hierarchical clustering with the top 50 genes associated with PCI and clustered the genomic discovery cohort into two groups of patients with distinct gene expression patterns (Figure 3A). Significant difference was found in the distributions of PCI between the two clusters (t-test $P < 0.0001$). Indeed, a cutoff of 0.77 in PCI achieved the highest accuracy of 82% for separating the two groups identified by unsupervised clustering (Figure 3B). This was consistent with the cutoff value of 0.8 identified earlier using Kaplan-Meier analysis of overall survival (Figure 2A).

Independent validation on gene expression datasets

The nearest shrunken centroid classifier achieved an accuracy of 77.5% for classifying low versus high-PCI on genomic discovery cohort. This radiogenomic classifier significantly stratified patients in 3 independent genomic validation cohorts and the meta-cohort in terms of overall survival (Figure 4, log rank P ranging between <0.0001 and 0.019), whereas the result for the smallest individual cohort (GSE37745, $n = 130$) trended toward statistical significance (log rank $P = 0.066$). On multivariate analysis, PCI remained a significant predictor of overall survival in the meta-cohort ($P = 0.0007$), independent of patient age, gender, and tumour stage (Table 3).

Discussion

In this study, we explicitly quantified relations between tumour and its neighbouring pleura on CT images by a pleural contact index and demonstrated its prognostic significance in independent cohorts of stage I NSCLC. To further validate the proposed imaging marker, we constructed a radiogenomic classifier that links gene expression with pleural contact index

on CT. While providing indirect evidence, this did allow us to confirm its prognostic value on large-scale, multiple independent stage I NSCLC cohorts with gene expression data.

Currently, most patients with stage I NSCLC do not receive adjuvant systemic treatment after curative surgery or radiotherapy because several large randomised studies have failed to show a survival benefit[35; 36]. A major issue has been the toxicity associated with chemotherapy, which may outweigh the potential benefit for many low-risk patients. Biomarkers that can reliably predict disease recurrence and survival are needed to guide the management of lung cancer. Early stage NSCLC patients with a higher PCI may have higher risks of metastasis and deaths, and might be potential candidates for receiving adjuvant chemotherapy. The clinical utility of PCI requires prospective validation in larger cohorts. Noninvasive prognostic imaging markers such as PCI would provide important benefits for longitudinal follow-up care of patients, or for inoperable patients treated with radiotherapy as tissue specimens in biopsies are mostly used up for diagnostic purposes and insufficient tissue are left for comprehensive molecular profiling.

We found that tumour volume was significantly associated with OS in the imaging validation cohort, but not in the imaging discovery cohort. One potential explanation for this discrepancy could be the individualised treatment protocol used in the imaging discovery cohort. In the lung tumour volume-adapted SABR dosing strategy, smaller tumours (<12 mL) received smaller radiation dose with BED<100 Gy, and larger tumours received larger dose with BED>100 Gy. On the other hand, PCI was significantly associated with OS in the imaging discovery cohort, but not in the imaging validation cohort when adjusting for tumour volume and other variables. This could be attributed to a moderate correlation between PCI and tumour volume (Pearson correlation coefficient = 0.53) in the imaging validation cohort, while the correlation was low at 0.22 in the imaging discovery cohort.

Several studies have investigated the potential prognostic value of the presence or absence of plural attachment in lung cancer based on radiologists' visual assessment of CT scans. Wang *et al.* found that plural attachment was associated with poor survival in resected lung adenocarcinoma[37; 38]. We did not observe this relation in our stage I NSCLC cohort treated with SABR (log rank $P=0.14$) or the imaging validation cohort treated with radiation therapy (log rank $P=0.11$). This difference could be due to different study populations in terms of tumour stage, histology, and treatment etc. When we further quantified the degree of pleural contact, a cutoff of 0.8 did show a significant difference in overall survival and distant metastasis in our stage I SABR cohort, and also stratified patients in an independent stage I NSCLC cohort. Thus, quantitative analyses of pleural contact on CT provided more granular information, leading to improved prognostic value.

There are important distinctions between our study and others with the goal of noninvasive evaluation of pathological pleural invasion. For example, authors in a recent study[27] showed a good 'diagnostic' accuracy for differentiating visceral from parietal pleural invasion based on tumour-pleura interface drawn free-hand. However, whether it is related to prognosis was not clear, nor was the biological underpinnings assessed in the previous study[27]. Here, we explicitly evaluated the prognostic relevance of PCI in the context of predicting recurrence and survival in early stage NSCLC, and identified its association with

ECM remodelling. Because both imaging cohorts consist of patients who underwent radiotherapy, surgical specimens were not available for these patients and thus we could not assess the relation between PCI and pathological pleural invasion. We determined the optimal cutoff for survival stratification at 0.8, which is different from that of 0.9 used in a diagnostic setting[27]. This difference in cutoff, albeit small, suggests that some tumours with visceral pleural invasion (associated with lower PCI than parietal invasion) might harbour aggressive disease and portend poor prognosis, which is consistent with a recent meta-analysis of 22 studies[18].

Most previous radiogenomic studies[39–45] attempted to find imaging predictors of a certain genomic feature (*e.g.*, EGFR mutation) using a top-down approach. By contrast, we adopted a bottom-up approach to identify the molecular features (ECM remodelling) correlated with a specific imaging phenotype, *i.e.*, CT finding of pleural contact. ECM is a major component of the tumour microenvironment, which modulates cancer cell mobility, migration and signalling[10–13]. ECM degradation is a prerequisite step for malignant cells to invade and metastasise[46]. The enrichment of genes in biological processes such as ECM organisation and collagen catabolic process suggested an association between pleural contact and a metastasis-prone tumour microenvironment. Additionally, lines of evidence have shown that aberrant ECM can lead to increased stability and bioavailability of growth factors and cytokines in tumour microenvironment resulting in enhanced tumour cell proliferation[47]. Consistently, the enrichment of biological processes such as immune response and leukocyte migration implies that pleural contact might correlate with an unbalanced cytokine context in lung cancer.

While the pleural contact index seems to provide useful prognostic information in an overall population, it does not distinguish among isolated tumours without pleural contact (PCI=0). For those tumours, other types of image features such as tumour size, morphology or texture[22; 37; 38; 48–53] may provide additional information. In future work, it may be beneficial to combine these image features to further improve prognostic accuracy.

Limitations of the study should be noted. First, our work identified correlative but not causative relations between molecular and imaging phenotypes. Future investigation should be conducted to confirm the molecular mechanisms that may be driving the imaging behaviour. Second, intratumoural heterogeneity[54] may confound our radiogenomic analysis, but this is not unique to our study and affects all genetic studies. Nevertheless, given the extensive evidence of ECM remodelling in cancer invasion, the finding of its association with an image surrogate of pleural invasion seems reasonable. Finally, while we included multiple independent validation cohorts, our study was retrospective in nature. Prospective studies are needed to confirm the findings of our study.

In summary, the pleural contact index evaluated on CT is associated with ECM remodelling and may serve as a noninvasive prognostic image marker. If validated in prospective studies, the imaging biomarker might be used to guide adjuvant therapy in early stage NSCLC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key points

1. A quantitative pleural contact index (PCI) predicts survival in early stage NSCLC.
2. PCI is associated with extracellular matrix organisation and collagen catabolic process.
3. A multi-gene surrogate of PCI is an independent predictor of survival.
4. PCI can be used to noninvasively identify patients with poor prognosis.

Figure 1.
Illustration of the study design.

Figure 2.

Kaplan-Meier curves of overall survival (A) and freedom from distant metastasis (B) in the imaging discovery cohort, overall survival in the imaging validation cohort (C), overall survival within clinical stage IA (tumour size <3 cm) and IB (tumour size >3 cm) subgroups in the combined imaging discovery and validation cohorts (D–E) stratified by PCI. The cut-off value was obtained from the discovery cohort and fixed for the validation cohort.

Figure 3. (A) Hierarchical clustering of top 50 genes associated with PCI in the genomic discovery cohort revealed two distinct clusters. (B) Boxplot of the pleural contact index for the respective clusters showed significantly different distributions.

Figure 4.

Prognostic performances of the gene-expression based imaging phenotype model on 4 independent genomic validation cohorts (A) GSE30219, (B) GSE31210, (C) GSE68645, (D) GSE37745, and (E) meta-cohort.

Figure 5.

Kaplan-Meier curves of overall survival in the imaging discovery cohort within clinical stage IA and IB subgroups (A–B) and in the imaging validation cohort within clinical stage IA and IB subgroups (C–D).

Table 1

Clinical and Treatment Information for the Imaging Discovery Cohort (117 patients) and Imaging Validation Cohort (88 patients)

	Discovery Cohort (n=117)	Validation Cohort (n=88)	P Value [†]
Age (y)			0.491
Median	75 (42–92)	74 (49–91)	
Mean ± standard deviation	74.2 ± 10.1	73.1 ± 9.0	
Gender			0.001
Male	53 (45.3)	60 (68.2)	
Female	64 (54.7)	28 (31.8)	
Clinical stage			< 0.001
IA	85 (72.6)	39 (44.3)	
IB	32 (27.3)	49 (55.7)	
Max tumour diameter (cm)			< 0.001
x < 2	39 (33.3)	10 (11.4)	
2 x < 3	46 (39.3)	29 (33.0)	
x ≥ 3	32 (27.4)	49 (55.6)	
Central vs. Peripheral[‡]			0.15
Central	43 (36.8)	41 (46.6)	
Peripheral	74 (63.2)	47 (53.4)	
Histologic type			< 0.001
Adenocarcinoma	71 (60.7)	10 (11.4)	
Squamous cell carcinoma	31 (26.5)	21 (23.8)	
Other	4 (3.4)	30 (34.1)	
Unknown	11 (9.4)	27 (30.7)	
Pleural Attachment[✓]			0.24
No	40 (34.2)	38 (43.2)	
Yes	77 (65.8)	50 (56.8)	
Local failure			
No	109 (93.2)	NA	
Yes	8 (6.8)	NA	
Regional failure			
No	108 (92.3)	NA	
Yes	9 (7.7)	NA	
Distant metastasis			
No	97 (76.9)	NA	
Yes	20 (23.1)	NA	
Overall survival			0.01
Alive	68 (58.1)	35 (39.8)	

	Discovery Cohort (n=117)	Validation Cohort (n=88)	P Value [†]
Deceased	49 (41.9)	53 (60.2)	

Note: Unless otherwise indicated, data are number of patients, with percentage in parentheses.

[†] Statistical comparison between two cohorts was computed with Wilcoxon rank sum test and χ^2 test for continuous and categorical variables, respectively.

[‡] Tumours within 2 cm of heart, great vessels, trachea, proximal bronchial trees or other mediastinal structures as central, otherwise peripheral.

[↓] Pleural attachment is a binary descriptor of tumour (not attached versus attached to pleura).

Table 2

Cox univariate and multivariate analysis in Imaging Discovery and Validation Cohorts

Variables	Imaging Discovery Cohort			Imaging Validation Cohort		
	DMFS	Univariate	Multivariate	Univariate	Multivariate	OS
Pleural Contact Index[*]						
	.0059 (HR 3.62 95% CI 1.449–9.085)	.0037 (HR 2.388 95% CI 1.327–4.297)	.0053 (HR 8.310 95% CI 1.876–36.818)	.0203 (HR 1.985 95% CI 1.113–3.540)	.0411 (HR 2.721 95% CI 1.041–7.112)	.3538
Pleural Contact Arc length[‡]						
	.1420	.1480	.1119	.0004 (HR 1.006 95% CI 1.003–1.009)	.8169	.7631
Pleural attachment[‡]						
	.0140 (HR 4.694 95% CI 1.367–16.12)	.1410	.3074	.1120	.9035	.9115
Max tumour diameter						
	.0153 (HR 1.775 95% CI 1.116–2.821)	.2090	.0035 (HR 3.347 95% CI 1.487–7.538)	.0001 (HR 1.240 95% CI 1.112–1.383)	.3236	.9086
Gross tumour volume						
	.2710	.6390	.2557	.0001 (HR 1.009 95% CI 1.006–1.012)	.3588	.0174 (HR 1.010 95% CI 1.002–1.018)
Central vs. Peripheral 						
	.0778	.3230	.0248 (HR 3.117 95% CI 1.155–8.416)	.2563	.6150	.9452
Histology[§]						
	.215	.7210	.0607	.7138	.5710	.1378
Age						
	.5120	.0076 (HR 1.043 95% CI 1.011–1.076)	.7278	.0160 (HR 1.045 95% CI 1.008–1.083)	.0544	.2831
Gender						
	.8340	.6590	.5369	.7630	.2930	.7724
Biological effective dose						

Variables	Imaging Discovery Cohort			Imaging Validation Cohort		
	Univariate		DMFS	Multivariate		OS
	OS	DMFS	OS	OS	OS	N/A
	.3890	.6350	.3269	N/A	N/A	

Note: Data are p-value of cox regression models, with hazard ratio (HR) and 95% confidence interval (CI).

* Pleural contact index was dichotomised into binary variables (>0.8 vs. 0.8).

[†] Pleural contact arc length is the length of the tumour boundary attached to pleura.

[‡] Pleural attachment is a binary descriptor of tumour (not attached versus attached to pleura).

■ Tumours within 2 cm of heart, great vessels, trachea, proximal bronchial trees or other mediastinal structures as central, otherwise peripheral.

[§] histology information was compared between squamous and non-squamous groups.

Unless otherwise indicated, variables are continuous.

Table 3

Univariate and Multivariate Cox Regression Analyses in the Meta Gene Expression Cohort

Predictors	Univariate		Multivariate	
	P-Value	Hazard Ratio	P-Value	Hazard Ratio
Predicted PCI (>0.8 vs. 0.8)	<0.0001	1.634	0.0007	1.456
Age (>65yr vs. 65yr)	<0.0001	2.168	<0.0001	2.007
Gender (Male vs. Female)	0.0004	1.517	0.0015	1.453
Stage (IB vs. IA)	<0.0001	1.661	0.0039	1.381