

Computed tomography-based quantification of intra-tumoral heterogeneity for predicting treatment response to concurrent chemoradiotherapy in non-small cell lung cancer: a multicenter retrospective study

Benlan Li^{1,2,3#}, Zhongjun Huang^{1,2,4#}, Yong Hu⁵, Bo Du⁵, Youlong Mo⁵, Tianchu He⁶, Mingdan Zhao⁷, Ya Li^{1,2,3}, Min Zhang^{1,2,3}, Ji Xia^{1,2,3}, Minfang Wang^{1,2,3}, Xian Liu^{1,2,8}, Fangyang Lu², Lingyun Wang⁸, Shengfa Su^{1,3}, Jie Peng^{1,2}

¹Department of Oncology, School of Clinical Medicine, Guizhou Medical University, Guiyang, China; ²Department of Oncology, The Second Affiliated Hospital, Guizhou Medical University, Kaili, China; ³Department of Oncology, The Affiliated Hospital of Guizhou Medical University, Guiyang, China; ⁴Department of Oncology, The Xingyi People's Hospital, Xingyi, China; ⁵Department of Oncology, Guiyang Pulmonary Hospital, Guiyang, China; ⁶Department of Oncology, Qiandongnan Prefecture People's Hospital, Kaili, China; ⁷Department of Oncology, Qiannan Prefecture Hospital of Traditional Chinese Medicine, Duiyun, China; ⁸Department of Oncology, Guiyang First People's Hospital, Guiyang, China

Contributions: (I) Conception and design: B Li, Z Huang, J Peng; (II) Administrative support: S Su, J Peng; (III) Provision of study materials or patients: Y Hu, B Du, Y Mo, T He, M Zhao, L Wang; (IV) Collection and assembly of data: B Li, Z Huang, Y Li, M Zhang, J Xia, X Liu, M Wang; (V) Data analysis and interpretation: B Li, Z Huang; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work as co-first authors.

Correspondence to: Jie Peng, MD. Department of Oncology, School of Clinical Medicine, Guizhou Medical University, Guiyang, China; Department of Oncology, The Second Affiliated Hospital, Guizhou Medical University, 3 Rehabilitation Road, Kaili 556000, China. Email: sank44@sina.com; Shengfa Su, MD. Department of Oncology, School of Clinical Medicine, Guizhou Medical University, Guiyang, China; Department of Oncology, The Affiliated Hospital of Guizhou Medical University, 9 Beijing Road, Yunyan District, Guiyang 550004, China. Email: sushengfa2005@163.com.

Background: Concurrent chemoradiotherapy (CCRT) constitutes a cornerstone of treatment for non-small cell lung cancer (NSCLC). However, NSCLC's intrinsic heterogeneity yields variable therapeutic responses. Noninvasive, quantitative assessment of intra-tumoral heterogeneity (ITH) may improve prediction of treatment efficacy. This study sought to derive ITH metrics from pretreatment contrast-enhanced computed tomography (CT) and to assess their prognostic value for outcomes following CCRT in NSCLC patients.

Methods: In this multicenter, retrospective study, pretreatment CT scans from NSCLC patients undergoing CCRT between January 1, 2019, and December 31, 2023 were allocated to training (n=132) and validation (n=32) cohorts. Conventional radiomic features were extracted from the tumor region of interest. A Gaussian mixture model (GMM) determined three optimal tumor subregions, from which ecological diversity metrics were computed in Python (v3.6) and subjected to least absolute shrinkage and selection operator (LASSO) regression. Support vector machine (SVM) generated both the ITH and C-radiomics classifiers. Multivariable logistic regression then integrated clinical, C-radiomics, and ITH predictors into a unified prognostic model, whose discriminative ability was quantified by the area under the receiver operating characteristic curve (AUC). Patients were dichotomized by ITH and combined-model thresholds to evaluate stratification of predicted response.

Results: Thirteen ecological diversity metrics informed the ITH classifier. The ITH model achieved AUCs of 0.78 [95% confidence interval (CI): 0.70–0.86] in training and 0.77 (95% CI: 0.59–0.94) in validation. Incorporation of clinical and radiomic features yielded a combined model with AUCs of 0.92 (95% CI: 0.86–0.98) and 0.87 (95% CI: 0.72–1.00), respectively. According to the cutoff values of the ITH and combined models, patients were stratified (ITH model: 0.65; combined model: 0.53, 0.86), and significant differences in progression-free survival (PFS) and overall survival (OS) were observed in the high-risk and low-risk groups (P<0.05).

Conclusions: The CT-based ITH model exhibited robust predictive accuracy, surpassing standalone clinical and conventional radiomic models. A unified model combining clinical, radiomic, and ITH features further enhanced prognostic precision for CCRT response in NSCLC. Both models successfully stratified patients by PFS and OS risk, underscoring their potential to inform personalized therapeutic decision-making.

Keywords: Radiomics; intra-tumoral heterogeneity (ITH); non-small cell lung cancer (NSCLC); concurrent chemoradiotherapy (CCRT); treatment response

Submitted Feb 26, 2025. Accepted for publication May 16, 2025. Published online Sep 25, 2025.

doi: 10.21037/tcr-2025-438

View this article at: <https://dx.doi.org/10.21037/tcr-2025-438>

Introduction

Background

Lung cancer is classified into two principal types: non-small cell lung cancer (NSCLC) and small cell lung

cancer (SCLC). NSCLC accounts for the majority of lung malignancies (1), and approximately 60 percent of NSCLC cases are identified at locally advanced or metastatic stages (2). Among early-stage and locally advanced NSCLC patients, roughly 75–80 percent are ineligible for curative resection (3).

For early-stage, inoperable NSCLC, stereotactic body radiotherapy (SBRT) is the recommended standard treatment (4,5). In locally advanced NSCLC, concurrent chemoradiotherapy (CCRT) remains the principal therapeutic approach (6–8). Reportedly, the 5-year survival rate for individuals undergoing CCRT was merely 15% (9), the poor survival rates in NSCLC chiefly stem from its aggressive behavior and complex tumor biology (10). Intra-tumoral heterogeneity (ITH) and the tumor microenvironment (TME) drive disease progression and undermine therapeutic efficacy (11). Variations in cellular composition and spatial architecture within individual tumors yield disparate responses to CCRT, with nearly 73% of patients experiencing locoregional failure after radiotherapy (12,13).

Tumor heterogeneity complicates clinical decision-making and longitudinal management. Early and precise identification of high-risk patients, coupled with timely adaptation of treatment regimens, could curtail disease progression and reduce mortality—an imperative in precision oncology. Advances in gene sequencing technology and pathologic histology have led to the identification of several molecular biomarkers for a wide range of cancer types (14–16), contributing to our understanding of the diverse origins of tumor cells (17). However, biomarker discovery predominantly relies on tissue biopsies, which fail to encompass the full spectrum of intra-tumoral diversity (18). Hence, there is an urgent need to develop noninvasive, comprehensive diagnostic modalities.

Highlight box

Key findings

- We constructed a machine-learning-driven model of intra-tumoral heterogeneity (ITH) using pre-treatment contrast-enhanced computed tomography scans to predict non-small cell lung cancer patients' responses to concurrent chemoradiotherapy (CCRT).
- The model achieved robust discrimination in the training cohort [area under the receiver operating characteristic curve (AUC) =0.78] and maintained performance in the validation cohort (AUC =0.77).
- Incorporating intra-tumoral ecological-diversity metrics alongside clinical variables and conventional radiomic signatures further improved accuracy (AUC =0.92 training, 0.87 validation).

What is known and what is new?

- Previous studies have shown that radiomics predict treatment response and prognosis in lung-cancer radiotherapy cohorts.
- However, these investigations relied on whole-tumor radiomics and overlooked subregional heterogeneity in CCRT response.
- In this study, we present a novel subregional-radiomics framework to quantify ITH comprehensively, thereby enhancing predictive precision.

What is the implication, and what should change now?

- Our results indicate that combining ecological-diversity metrics with clinical and radiomic data can refine therapeutic decision-making.
- This model may assist clinicians in identifying non-responders to CCRT, facilitating personalized treatment strategies.
- Future work should pursue external validation in larger prospective cohorts and adopt automated segmentation methods to bolster model robustness.

Rationale and knowledge gap

Lung tumor radiomics entails the automated extraction of quantitative features—both first-order and higher-order metrics—that characterize signal intensity distributions and spatial relationships within medical images (18). Specific radiomic signatures have been correlated with imaging texture heterogeneity and prognostic outcomes across cancer types (19–21). A principal benefit of radiomics lies in its noninvasive evaluation of the entire tumor volume, overcoming sampling bias inherent to biopsy-based assays (22). As radiomics and artificial intelligence continue to evolve to provide new research directions for predicting tumor treatment response, machine learning techniques have emerged as an analytic tool for medical imaging and data sequencing for diagnostic, prognostic, and efficacy assessment of patients with various cancer types (23–29). Radiomics analysis, when employing machine learning algorithms, is considered to enhance the performance of predictive models. Recent evidence has demonstrated encouraging results using radiomics features to develop an ITH-based quantitative measure for assessing treatment response in cancer (30). The application of image texture heterogeneity features derived from CT images, in conjunction with machine learning, to predict the effectiveness of CCRT for NSCLC has yet to be reported.

Objective

This study aimed to establish a quantitative indicator of ITH based on the pre-therapy enhanced CT scan results, construct a prediction model for ITH using machine learning methods, and evaluate whether combining this quantitative measure with clinical and C-radiomics features in a single model can predict the efficacy of CCRT for NSCLC. Additionally, we investigated the correlation between ITH and progression-free survival (PFS) as well as overall survival (OS). We present this article in accordance with the TRIPOD reporting checklist (available at <https://tcr.amegroups.com/article/view/10.21037/tcr-2025-438/rc>).

Methods

Patients

We retrospectively acquired pre-treatment CT scans from patients with NSCLC who received CCRT at multiple centers between January 1, 2019, and December 31, 2023. The dataset utilized in this study comprised 164

patients who had undergone CCRT across six medical centers: The Second Affiliated Hospital, Guizhou Medical University, Guiyang Pulmonary Hospital, Qiandongnan Prefecture People's Hospital, Qiannan Prefecture Hospital of Traditional Chinese Medicine, Qiannan People's Hospital, Guiyang First People's Hospital. Participants were separated into two distinct categories: a training group consisting of 132 individuals and a validation group with 32 individuals. Inclusion criteria are as follows: (I) histologically confirmed NSCLC; (II) CT-enhanced images of the chest in the month before therapy; (III) receiving the standard conventional dose-split radiotherapy regimen and concurrent chemotherapy (no previous specialized cancer treatments before this study); and (IV) Karnofsky performance status (KPS) score ≥ 70 . The following are the criteria for exclusion: (I) the presence of other malignant tumors; (II) radical surgery, targeting, and chemotherapy prior to chemoradiotherapy; (III) poor quality of CT image; (IV) missing data from follow-up visits (*Figure 1*). The CCRT regimen included a target-area dose of 60–66 Gy/30–33 fractions. The chemotherapy regimens used in this study were the squamous cancer chemotherapy regimen (paclitaxel 50 mg/m², d1, weekly + carboplatin AUC2, d1, weekly) and non-squamous cancer chemotherapy regimen (pemetrexed 500 mg/m², d1, 3 weeks + cisplatin 75 mg/m², d1, 3 weeks). In this study, we defined CCRT as two to four cycles of chemotherapy administered concurrently with thoracic radiotherapy. The study was conducted in accordance with the Declaration of Helsinki and its subsequent amendments. The study was approved by The Second Affiliated Hospital, Guizhou Medical University Ethics Committee (approval No. 2020-LS-03) and individual consent for this retrospective analysis was waived. The other institutions were also informed and approved the study.

The primary outcome of this study was the response to treatment within 3 months post-CCRT, while the secondary outcomes were PFS and OS of patients. Approximately 3 months after CCRT, treatment response was evaluated by imaging according to RECIST 1.1: complete or partial remission was considered an objective response.

Study design

The study design is presented in *Figure 2*. It comprised the following procedures: image acquisition and outlining of the region of interest (ROI), subregion clustering, extraction and selection of features, constructing and validating

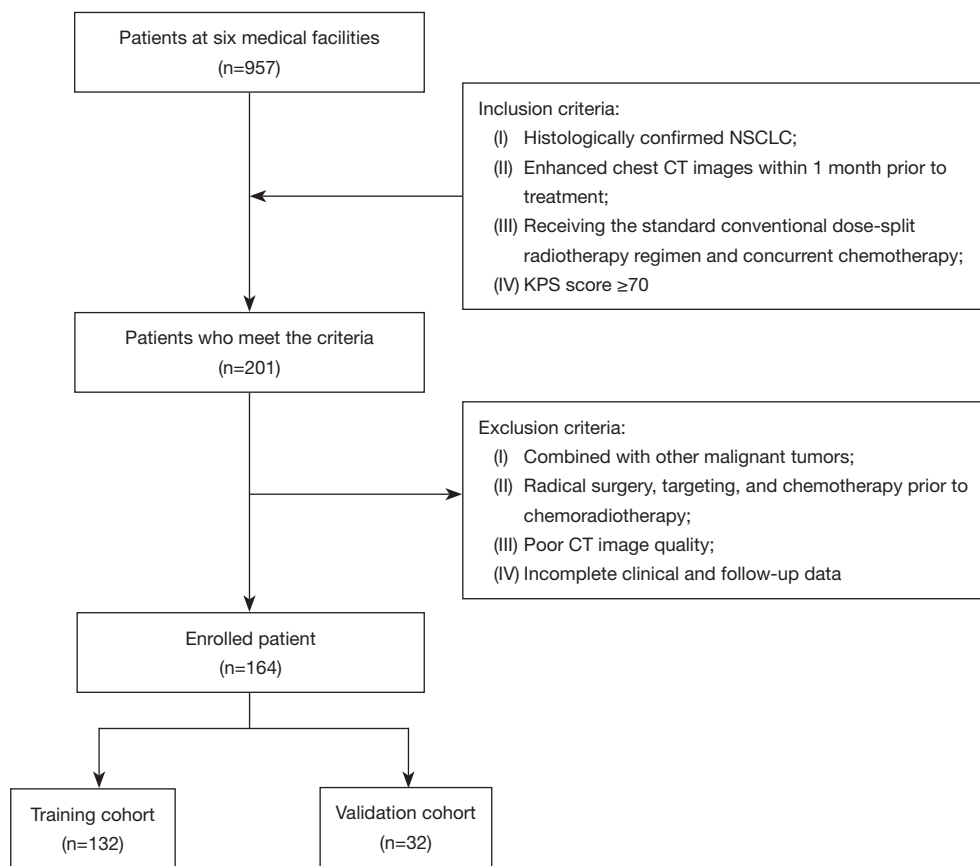


Figure 1 The flowchart of inclusion and exclusion criteria of patients. CT, computed tomography; KPS, Karnofsky performance status; NSCLC, non-small cell lung cancer.

predictive models, as well as survival analysis. The details of the study procedures are as follows:

- ❖ Step 1: comparison-enhanced CT imaging was performed in a laboratory setting using a multislice helical CT scanner (31). CT images were standardized, and the ROIs were outlined using ITK-SNAP software (version 3.8.0; <http://www.itksnap.org>) by an individual with 3 years of experience, and subsequently reviewed by a senior radiologist.
- ❖ Step 2: to extract ITH features, we applied Bayesian information criterion (BIC)-based Gaussian mixture model (GMM) clustering to the tumor ROI, partitioning it into three subregions; conventional radiomic features were extracted from the entire, non-partitioned ROI.
- ❖ Step 3: we extracted 1,835 features from an individual subregion, for a total of 5,505 intra-tumoral ecological diversity features per sample. For comparison, we also extracted 1,835 features from the entire ROI using

C-radiomics methods.

- ❖ Step 4: for C-radiomics and intra-tumoral ecological diversity features, the superfluous features with strong correlation were removed after correlation analysis of all features. Subsequently, we employed the LASSO to diminish the number of dimensions and retain the most effective radiographic features.
- ❖ Step 5: upon calibrating the pertinent parameters, we use 11 machine learning algorithms such as support vector machine (SVM), RandomForest, and AdaBoost to generate receiver operating characteristic (ROC) curves and evaluated the area under the ROC curve (AUC), accuracy, sensitivity, and specificity across the various algorithms. We compared eleven candidate algorithms via cross-validation (*Table 1*) and ultimately selected the SVM, which demonstrated the highest AUC and accuracy on the training set and robust performance on validation, for final testing in the validation cohort.

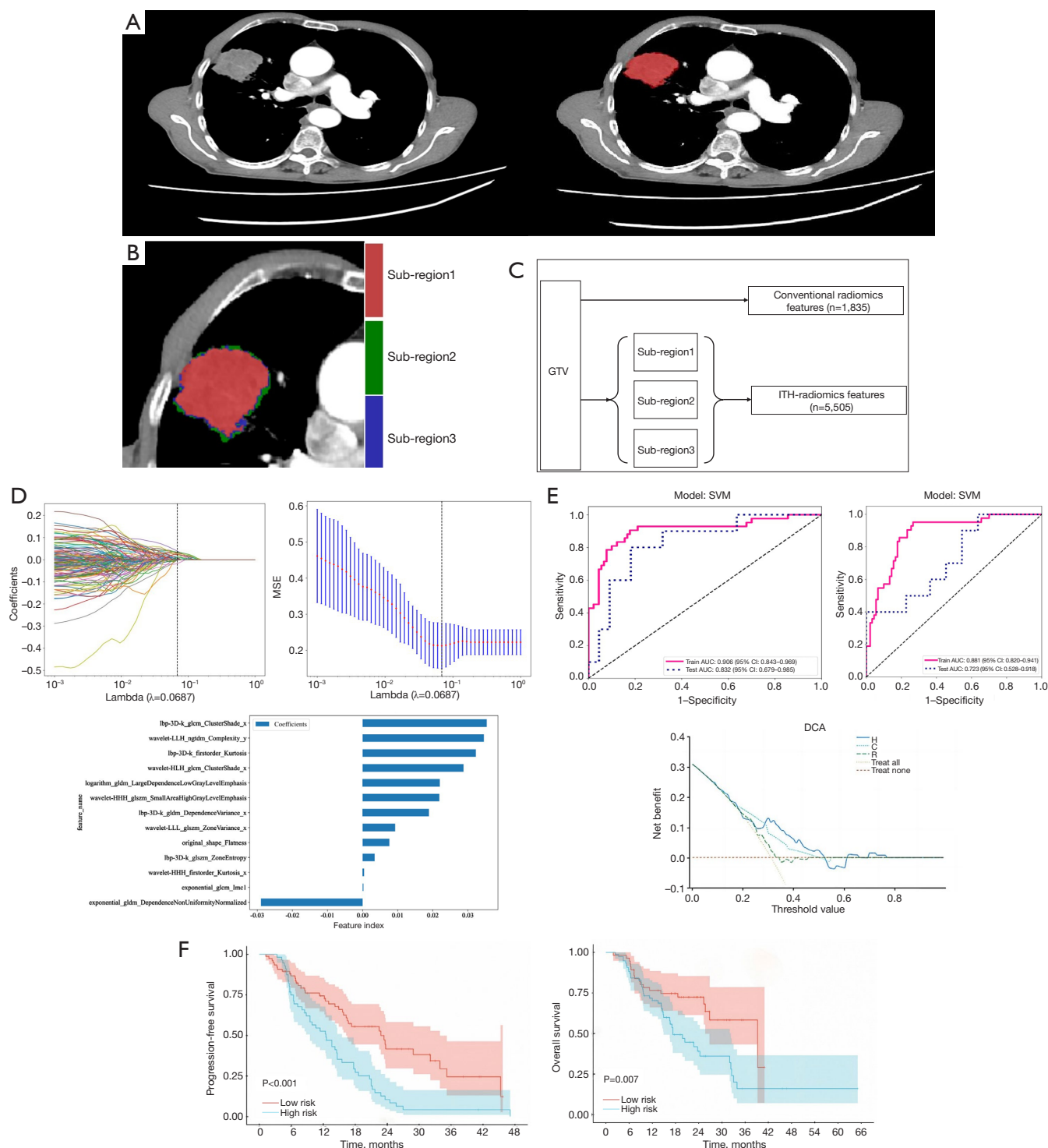


Figure 2 An illustration of the overall research design. (A) Acquisition of original images and ROI outlining; (B) segmentation of subregions; (C) feature extraction; (D) feature downscaling and selection; (E) model construction and decision curve analysis; (F) survival analysis. AUC, area under the receiver operating characteristic curve; C, clinical feature model; CI, confidence interval; CT, computed tomography; DCA, decision curve analysis; GTV, gross tumor volume; H, tumor heterogeneity feature model; ITH, intra-tumoral heterogeneity; KPS, Karnofsky performance status; NSCLC, non-small cell lung cancer; NSE, non-specific enolase; R, conventional radiomics feature model; SVM, support vector machine.

Table 1 Accuracy, specificity, sensitivity, and receiver operating characteristic curve of 11 machine learning algorithms

Model-name	Task	Accuracy	AUC	95% CI	Sensitivity	Specificity	PPV	NPV	Precision	Recall	F1	Threshold
LR	Train	0.72	0.84	0.76–0.91	0.86	0.66	0.54	0.91	0.54	0.86	0.66	0.27
	Test	0.69	0.76	0.57–0.95	0.80	0.64	0.50	0.88	0.50	0.80	0.62	0.16
NaiveBayes	Train	0.74	0.80	0.72–0.88	0.67	0.77	0.57	0.83	0.57	0.67	0.62	0.05
	Test	0.69	0.71	0.51–0.90	0.70	0.68	0.50	0.83	0.50	0.70	0.58	0.09
SVM	Train	0.86	0.91	0.85–0.97	0.91	0.83	0.72	0.95	0.72	0.91	0.80	0.26
	Test	0.78	0.81	0.65–0.98	0.70	0.82	0.64	0.86	0.64	0.70	0.67	0.32
KNN	Train	0.79	0.84	0.78–0.91	0.52	0.91	0.73	0.80	0.73	0.52	0.61	0.40
	Test	0.72	0.74	0.56–0.93	0.40	0.86	0.57	0.76	0.57	0.40	0.47	0.40
RandomForest	Train	0.99	1.00	0.99–1.00	0.98	1.00	1.00	0.99	1.00	0.98	0.99	0.50
	Test	0.59	0.65	0.44–0.85	0.60	0.59	0.40	0.77	0.40	0.60	0.48	0.30
ExtraTrees	Train	0.68	1.00	1.00–1.00	0.00	1.00	0.00	0.68	0.00	0.00	NaN	1.00
	Test	0.66	0.69	0.50–0.88	0.20	0.86	0.40	0.70	0.40	0.20	0.27	0.50
XGBoost	Train	0.99	1.00	1.00–1.00	0.98	1.00	1.00	0.99	1.00	0.98	0.99	0.59
	Test	0.72	0.74	0.55–0.92	0.80	0.68	0.53	0.88	0.53	0.80	0.64	0.25
LightGBM	Train	0.84	0.95	0.91–0.98	0.88	0.82	0.70	0.94	0.70	0.88	0.78	0.36
	Test	0.50	0.65	0.45–0.85	0.90	0.32	0.38	0.88	0.38	0.90	0.53	0.20
GradientBoosting	Train	0.91	0.98	0.97–0.99	0.93	0.90	0.81	0.96	0.81	0.93	0.87	0.33
	Test	0.69	0.69	0.50–0.88	0.70	0.68	0.50	0.83	0.50	0.70	0.58	0.33
AdaBoost	Train	0.87	0.94	0.91–0.98	0.86	0.88	0.77	0.93	0.77	0.86	0.81	0.48
	Test	0.59	0.68	0.49–0.87	0.8	0.50	0.42	0.85	0.42	0.80	0.55	0.44
MLP	Train	0.84	0.85	0.78–0.92	0.60	0.96	0.86	0.84	0.86	0.60	0.70	0.39
	Test	0.72	0.75	0.56–0.94	0.80	0.68	0.53	0.88	0.53	0.80	0.64	0.33

AUC, area under the receiver operating characteristic curve; CI, confidence interval; KNN, K-nearest neighbors; LightGBM, Light Gradient Boosting Machine; LR, logistic regression; MLP, multilayer perceptron; NaN, not a number; NPV, negative predictive value; PPV, positive predictive value; SVM, support vector machine.

- ❖ Step 6: further validation of the model was conducted. The patients' PFS and OS were analyzed using the intra-tumoral ecological diversity features model and the integrated model combining clinical data, C-radiomics, and intra-tumoral ecological diversity features.

Acquisition of CT images

All patients received 2 mL/kg of an iodine-containing contrast agent (50 mL: 33.9 g, 320 mg iodine/mL) using a high-pressure syringe with a 20 s scan delay. The CT scanning area ranged from the cricothyroid membrane

level to the lower edge of the 12th rib (2–3 cm margin). All images were acquired from a CT scanner. The CT scanning systems and parameters of the six medical centers are shown in [Table S1](#). Pretreatment CT images were downloaded in DICOM format from the imaging systems of the six medical centers.

Delineation of intra-tumoral subregions and generation of ecological diversity features

Subregions within tumors with radiomic features were clustered using a GMM. Using the BIC, we objectively determined the optimal number of clusters to represent

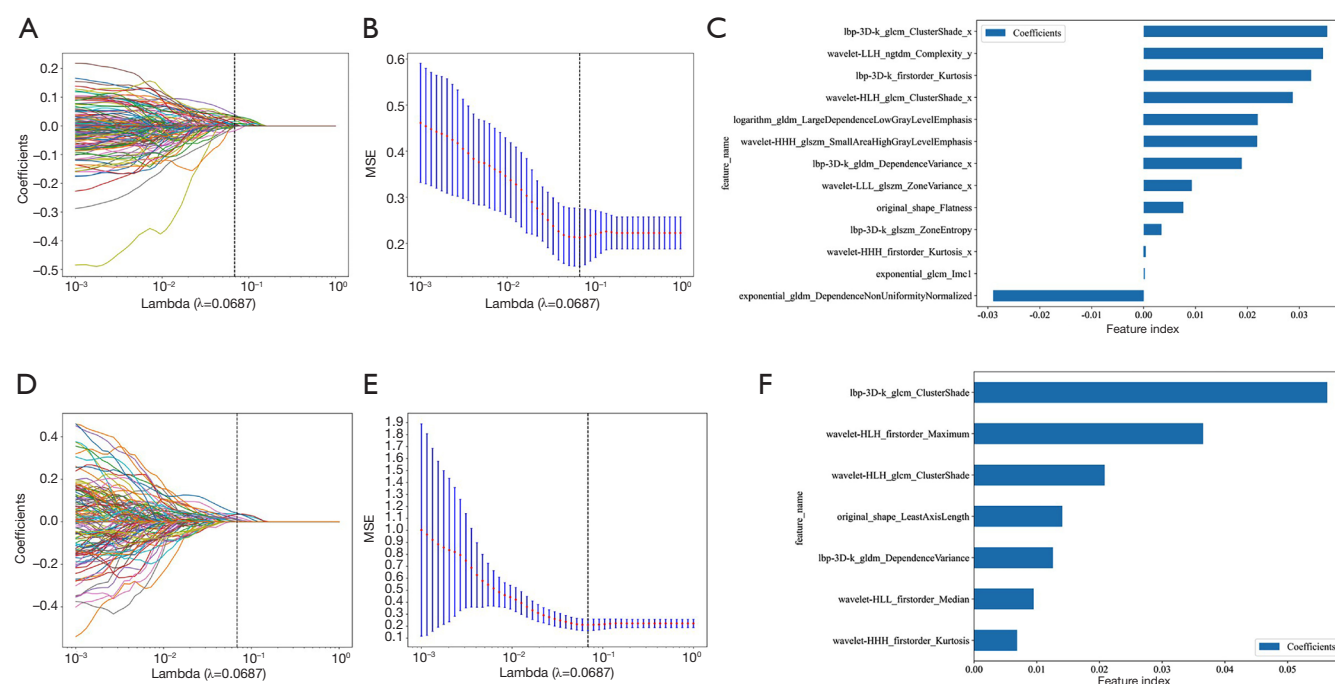


Figure 3 Radiomics feature selection and construction of radiomics models based on the LASSO algorithm. (A,D) Cross-validation coefficients of LASSO regression. (B,E) LASSO regression mean square error. (C,F) Weighting coefficients of selected features. (A-C) is intra-tumoral ecological diversity features (unmarked features indicate subregion 1, features marked with 'x' indicate subregion 2, and the feature marked with 'y' indicates subregion 3); (D-F) is conventional radiomics features. LASSO, least absolute shrinkage and selection operator; MSE, mean standard error.

tumor ecological diversity, selecting three clusters ($K=3$). Texture analysis and wavelet decomposition techniques were employed to quantify the volume, shape, intensity, and texture of each sub-region, resulting in 1,835 radiomic features (32). For the sake of comparison, 1,835 radiological features were derived from the entire tumor area and within-tumor subareas for each patient, employing the PyRadiomics package (version 3.1; <https://pyradiomics.readthedocs.io>) (33). Each radiomics set encompasses seven distinct categories of features: first-order, shape, grayscale covariance matrix, grayscale size region matrix, grayscale wander matrix, neighboring grayscale tone difference matrix, and grayscale correlation matrix features (the scanning parameters are detailed in Table S1).

Feature downscaling and predictive model development

Intra-tumoral ecological diversity features with high correlation (Pearson index >0.9) and conventional radiomics features were excluded to reduce radiomic feature redundancy. For conventional radiomics features, the

features were first standardized using a z-score number to exclude those with Pearson index >0.9 , and LASSO (five-fold cross-validation) was applied to reduce the dimensionality. For the intra-tumoral ecological diversity radiomics features, feature fusion was performed followed by standardization of the fused features. *T*-tests were applied to filter those with $P < 0.05$, followed by further exclusion of features with Pearson index >0.9 and LASSO application (five-fold cross-validation) to reduce the dimensionality. All feature-selection steps—including correlation filtering, Student's *t*-test, and LASSO with fivefold cross-validation—were performed exclusively on the training set, with no data from the validation cohort used. Ultimately, 13 intratumor diversity features and 7 C-radiomics features, both with *P* values below 0.05, were chosen for the development of the model (Figure 3). The ROC curves for intratumor ecological diversity and C-radiomics characterization are depicted in Figure S1. Univariate and multivariate logistic regression analyses were conducted to ascertain the associations of clinical variables with treatment efficacy, and two clinical variables were ultimately screened for use in

Table 2 Univariate and multivariate analyses for predicting the efficacy of concurrent radiotherapy in the training cohort

Variable	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P	OR (95% CI)	P
The number of chemotherapy cycles administered during CCRT				
<3	1.00 (reference)		1.00 (reference)	
≥3	0.32 (0.15–0.67)	0.003	0.34 (0.16–0.73)	0.006
NSE				
Normal	1.00 (reference)		1.00 (reference)	
Increase	0.38 (0.17–0.83)	0.02	0.41 (0.18–0.94)	0.03
Binary ITH index				
Low	1.00 (reference)		1.00 (reference)	
High	2.42 (1.14–5.11)	0.02	2.42 (1.14–5.11)	0.02

CCRT, concurrent chemoradiotherapy; CI, confidence interval; ITH, intra-tumoral heterogeneity; NSE, neuron-specific enolase; OR, odds ratio.

model development (*Table 2*).

Statistical analysis

Statistical analyses of clinical variables, model development, and survival comparisons were conducted with SPSS (version 25.0). Categorical data were evaluated via Chi-squared or Fisher's exact tests. Univariate and multivariate logistic regressions identified associations between patient characteristics and CCRT efficacy. Student's *t*-test and LASSO regression screened conventional radiomic signatures and intra-tumoral ecological diversity metrics. Based on feature selection results from the training cohort, the SVM algorithm was used to construct the (I) clinical characteristic (clinical) model; (II) C-radiomics features (C-radiomics) model; (III) intra-tumoral ecological diversity features (ITH) model; (IV) clinical characteristic + C-radiomics (clinical-radiomics) model; (V) clinical characteristic + intra-tumoral ecological diversity features (clinical-ITH) model; (VI) C-radiomics + intra-tumoral ecological diversity features (ITH-radiomics) model; and (VII) clinical characteristic + C-radiomics + intra-tumoral ecological diversity features (combined) model. Model performance was validated in an independent cohort. The Youden index determined optimal prediction thresholds. The continuous output of the ITH model was dichotomized at the optimal Youden index cutoff (0.65) derived from the training-set ROC; values above this threshold were classified

as high ITH and those below as low ITH, and these binary labels were then incorporated into a logistic regression. The PFS and OS in the high-risk and low-risk groups of the intra-tumoral ecological diversity features model and in the high-risk, intermediate-risk, and low-risk groups of the combined model were analyzed using the Kaplan-Meier method. The clinical applicability of the models was further assessed using decision curve analysis. LASSO regression and decision curve analyses were performed with the assistance of R software (version 4.3.1), where a P value below 0.05 was regarded as statistically significant.

Results

Characteristics of patients

The clinical characteristics and distribution of the 164 patients included in this study are shown in *Table 2*. In the training cohort (n=132) and validation cohort (n=32), 78 (59.09%) and 19 (59.38%) patients had a history of smoking, 108 (81.82%) and 25 (78.13%) were male, 75 (56.82%) and 18 (56.25%) had a pathological staging of adenocarcinoma, 74 (56.06%) and 21 (65.63%) had a tumor-node-metastasis (TNM) staging of stage III, and 50 (37.88%) and 10 (31.25%) had TNM staging of stage IV, respectively. Patients with high carcinoembryonic antigen levels were 50 (37.88%) in the training cohort and 9 (28.13%) in the validation cohort. Additionally, the

majority of patients in both cohorts were in stages T3–4 (training cohort: 71.21%; validation cohort: 65.63%), N2–3 (training cohort: 86.36%; validation cohort: 93.75%), and M0 (training cohort: 65.15%, validation cohort: 68.75%). In the training and validation cohorts, 43 (32.58%) and 12 (37.50%) individuals, respectively, achieved objective remission rates, including complete and partial remission, respectively. No significant variations were noted among the two groups of patients regarding gender, age, KPS score, metastasis status, clinical stages, and other factors ($P>0.05$) (Table 3).

Variables associated with CCRT efficacy in the training dataset

Univariate analysis revealed that the number of chemotherapy cycles administered during CCRT was significantly associated with outcome {odds ratio (OR), 0.32 [95% confidence interval (CI): 0.15, 0.67]; $P<0.05$ }, non-specific enolase (NSE) level [OR, 0.38 (95% CI: 0.17, 0.83); $P<0.001$], and binary ITH index [OR, 2.42 (95% CI: 1.14, 5.11); $P<0.05$] were significantly associated with efficacy in the training dataset. On multivariate analysis, the three factors (the number of chemotherapy cycles administered during CCRT, NSE

Table 3 Baseline characteristics of patients with NSCLC in the training cohort, validation cohort, and the total population

Characteristic	Total	Training cohort	Validation cohort	P
Sex				0.63
Female	31 (18.902)	24 (18.182)	7 (21.875)	
Male	133 (81.098)	108 (81.818)	25 (78.125)	
Age (years)				0.39
>60	66 (40.244)	51 (38.636)	15 (46.875)	
≤60	98 (59.756)	81 (61.364)	17 (53.125)	
Smoking status				0.98
Non-smoker	67 (40.854)	54 (40.909)	13 (40.625)	
Smoker	97 (59.146)	78 (59.091)	19 (59.375)	
KPS				0.48
>80	78 (47.561)	61 (46.212)	17 (53.125)	
≤80	86 (52.439)	71 (53.788)	15 (46.875)	
Pathological type				>0.99
Adenocarcinoma	93 (56.707)	75 (56.818)	18 (56.250)	
Squamous carcinoma	66 (40.244)	53 (40.152)	13 (40.625)	
Other	5 (3.049)	4 (3.030)	1 (3.125)	
Oligometastasis				0.52
No	125 (76.220)	102 (77.273)	23 (71.875)	
Yes	39 (23.780)	30 (22.727)	9 (28.125)	
Clinical T stage				0.54
T1–2	49 (29.878)	38 (28.788)	11 (34.375)	
T3–4	115 (70.122)	94 (71.212)	21 (65.625)	

Table 3 (continued)

Table 3 (continued)

Characteristic	Total	Training cohort	Validation cohort	P
Clinical N stage				0.40
N0–1	20 (12.195)	18 (13.636)	2 (6.250)	
N2–3	144 (87.805)	114 (86.364)	30 (93.750)	
Clinical M stage				0.70
M0	108 (65.854)	86 (65.152)	22 (68.750)	
M1	56 (34.146)	46 (34.848)	10 (31.250)	
Clinical stage				0.57
I–II	9 (5.488)	8 (6.061)	1 (3.125)	
III	95 (57.927)	74 (56.061)	21 (65.625)	
IV	60 (36.585)	50 (37.879)	10 (31.250)	
Invading mediastinal lymph nodes				0.22
No	45 (27.439)	39 (29.545)	6 (18.750)	
Yes	119 (72.561)	93 (70.455)	26 (81.250)	
Response status				0.60
CR + PR	55 (33.537)	43 (32.576)	12 (37.500)	
PD + SD	109 (66.463)	89 (67.424)	20 (62.500)	
Chemotherapy regimens				0.23
Platinum containing double drug	131 (79.878)	103 (78.030)	28 (87.500)	
Other	33 (20.122)	29 (21.970)	4 (12.500)	
The number of chemotherapy cycles administered during CCRT				0.33
<3	95 (57.927)	74 (56.061)	21 (65.625)	
≥3	69 (42.073)	58 (43.939)	11 (34.375)	
CEA				0.30
Normal	105 (64.024)	82 (62.121)	23 (71.875)	
Increase	59 (35.976)	50 (37.879)	9 (28.125)	
NSE				0.15
Normal	122 (74.390)	95 (71.970)	27 (84.375)	
Increase	42 (25.610)	37 (28.030)	5 (15.625)	
CYFRA21-1				0.39
Normal	61 (37.195)	47 (35.606)	14 (43.750)	
Increase	103 (62.805)	85 (64.394)	18 (56.250)	

CCRT, concurrent chemoradiotherapy; CEA, carcinoembryonic antigen; CR, complete response; CYFRA 21-1, cytokeratin 19 fragment; M, distant metastasis; N, regional lymph nodes; NSE, neuron-specific enolase; NSCLC, non-small cell lung cancer; PD, progressive disease; PR, partial response; SD, stable disease; T, tumor.

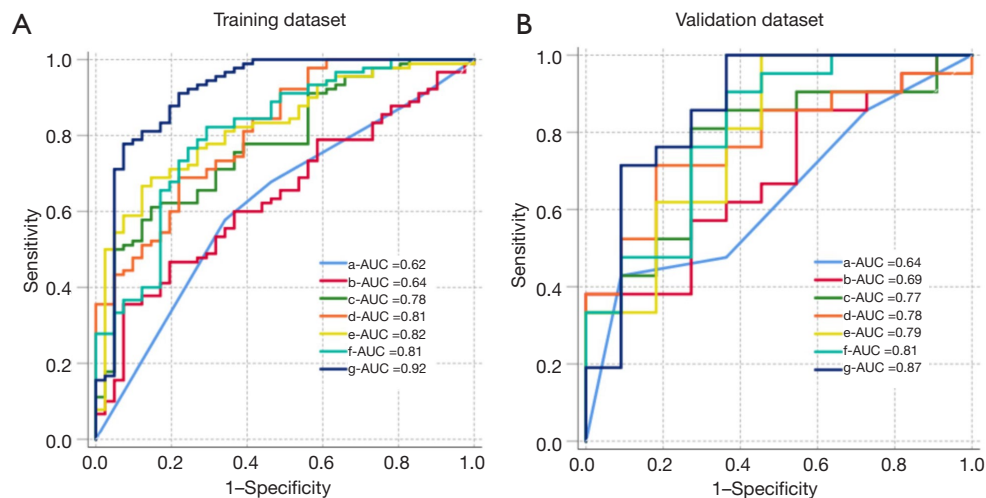


Figure 4 Performance of seven developed models for predicting the efficacy of concurrent chemoradiotherapy for NSCLC in all datasets. (A) Training dataset; (B) validation dataset. The AUC demonstrates that the model combining clinical features, conventional radiomics, and intra-tumoral ecological diversity features (ITH) (blue line) obtained the highest AUC values among all tested models, with AUCs of 0.92 and 0.87, respectively. (a) Clinical characteristic (clinical model); (b) C-radiomics features (C-radiomics model); (c) intra-tumoral ecological diversity features (ITH model); (d) clinical characteristic + C-radiomics (clinical-radiomics) model; (e) clinical characteristic + intra-tumoral ecological diversity features (clinical-ITH model); (f) c-radiomics + intra-tumoral ecological diversity features (ITH-radiomics model); (g) clinical characteristic + C-radiomics + intra-tumoral ecological diversity features (combined model). AUC, area under the receiver operating characteristic curve; ITH, intra-tumoral heterogeneity; NSCLC, non-small cell lung cancer.

level, and binary ITH index) retained their statistical significance and were thus considered independent predictors of treatment efficacy (Table 3).

Construction of predictive models and performance evaluation

A predictive model was built using the SVM algorithm, integrating the screened C-radiomics, intra-tumoral ecological diversity, and clinical features to further explore the predictive value of ITH and C-radiomics features on the efficacy of CCRT for NSCLC. In both cohorts, the AUCs of the ITH model were 0.78 (95% CI: 0.70–0.86) and 0.77 (95% CI: 0.59–0.94), respectively, which were better than those of C-radiomics (AUCs of 0.64 and 0.69 in both cohorts). Furthermore, the predictive value of the intra-tumoral ecological diversity features and C-radiomics features on NSCLC treatment efficacy was further investigated by integrating the clinical variables (first-time receipt of CCRT and NSE levels) and the ITH and C-radiomics features in the integrated model (clinical variables + conventional radiomics + ITH), which further enhanced the predictive accuracy, with AUCs reaching

0.92 (95% CI: 0.86–0.98) and 0.87 (95% CI: 0.72–1.00), respectively. The AUC for each model is displayed in Figure 4, and the accuracy, specificity, and sensitivity of the seven models are detailed in Table S2.

PFS and OS analysis of ITH models and joint models in the training and validation cohorts

The cut-off point of 0.65 was ascertained via the ROC curve analysis of the model that evaluates the ecological diversity within tumors. The optimal threshold was then set using the Youden index to segregate NSCLC patients into two categories: those with a low risk (cut-off point <0.65) and those with a high risk (cut-off point >0.65). For the combined model (g-model), the patients were classified into a low-risk (cut-off value <0.53), intermediate-risk (cut-off value: 0.53–0.86), and high-risk (cut-off value >0.86) groups. The correlation of the intra-tumoral ecological diversity features (ITH model) and the combined model with PFS and OS of the patients with NSCLC was optimized using the Kaplan-Meier method. The survival analyses revealed significant differences in PFS and OS between patients categorized into high-risk and low-risk groups according

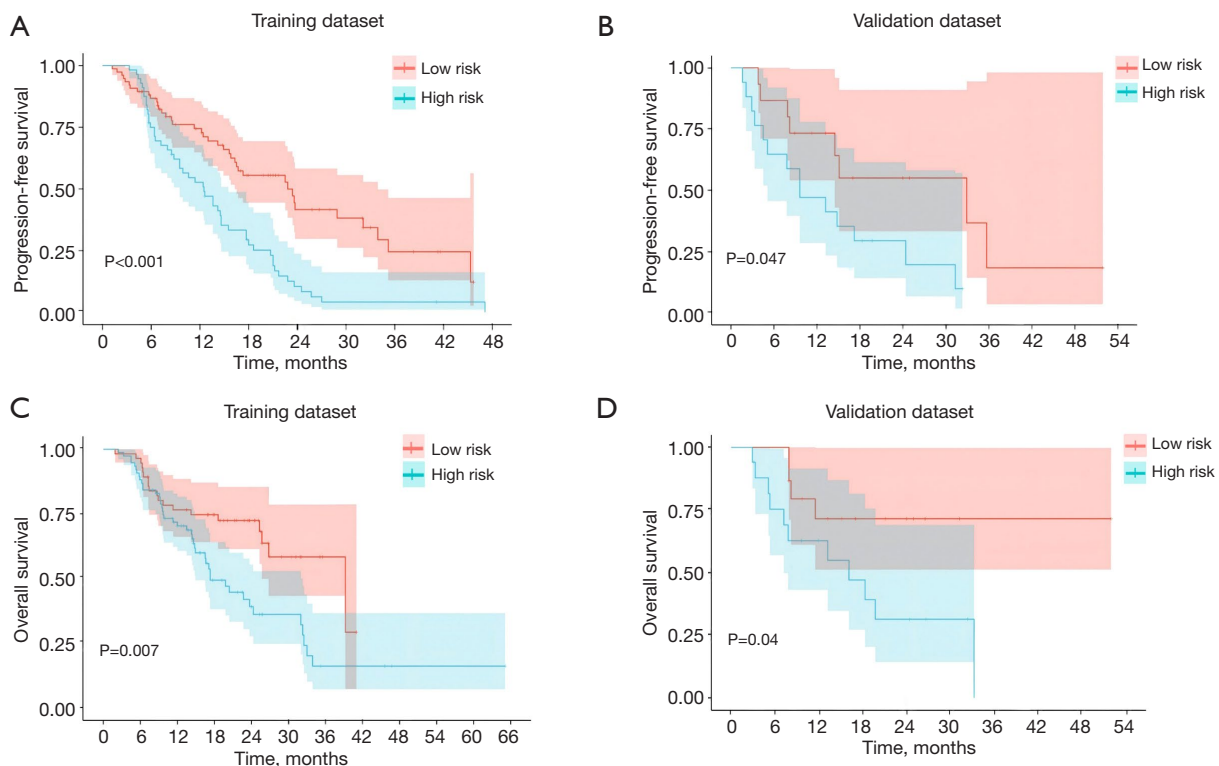


Figure 5 Survival curve analysis of progression-free survival (A,B) and overall survival (C,D) for patients in the high- and low-risk groups in the training and validation cohorts of the intra-tumoral ecological diversity features model.

to the cut-off value of 0.65 ($P < 0.05$) (Figure 5). Similarly, in the combined model, significant disparities in PFS and OS were observed among the high-risk, intermediate-risk, and low-risk groups, with the worst outcomes observed in the high-risk group (Figure 6). On decision curve analysis, the combined model outperformed the other models in most threshold ranges and could make more accurate and reliable efficacy predictions for clinical applications (Figure S2).

Discussion

We performed subregion clustering and extracted numerous intra-tumoral ecological-diversity features from pretreatment contrast-enhanced CT scans of NSCLC patients receiving CCRT. Feature selection and ITH-prediction-model development employed LASSO and SVM algorithms. The intra-tumoral ecological-diversity-based model robustly forecasted CCRT efficacy, yielding an AUC of 0.78 in the test cohort and 0.77 in the validation cohort. Its discriminative performance surpassed that of conventional metrics. Moreover, integrating these ecological-diversity features with clinical variables

and traditional radiomics further enhanced predictive accuracy—achieving AUCs of 0.92 and 0.87 for the combined and paired feature sets, respectively. Finally, the ITH model stratified CCRT-treated NSCLC patients into distinct risk categories: those in the high-risk group exhibited significantly poorer prognosis ($P < 0.05$).

Identifying patients most likely to benefit from CCRT remains a focal point of investigation. Prior studies have uncovered various molecular biomarkers across multiple cancer types (types 3–4) (34,35). Yet, biopsy- or surgery-derived specimens sample only limited tumor regions, failing to capture the full spatial heterogeneity of tumor tissue. Consequently, methods that comprehensively characterize ITH are indispensable for refining NSCLC diagnostic and prognostic decision-making. Radiomics—by converting imaging data into high-dimensional, quantitative features—has emerged as a powerful tool for predicting treatment response and outcomes in lung-cancer radiotherapy (36–38). However, most investigations to date have analyzed whole-tumor radiomic signatures, overlooking the interplay between ITH and radiomic predictors of CCRT response. In one notable study, researchers developed a CT-based ITH

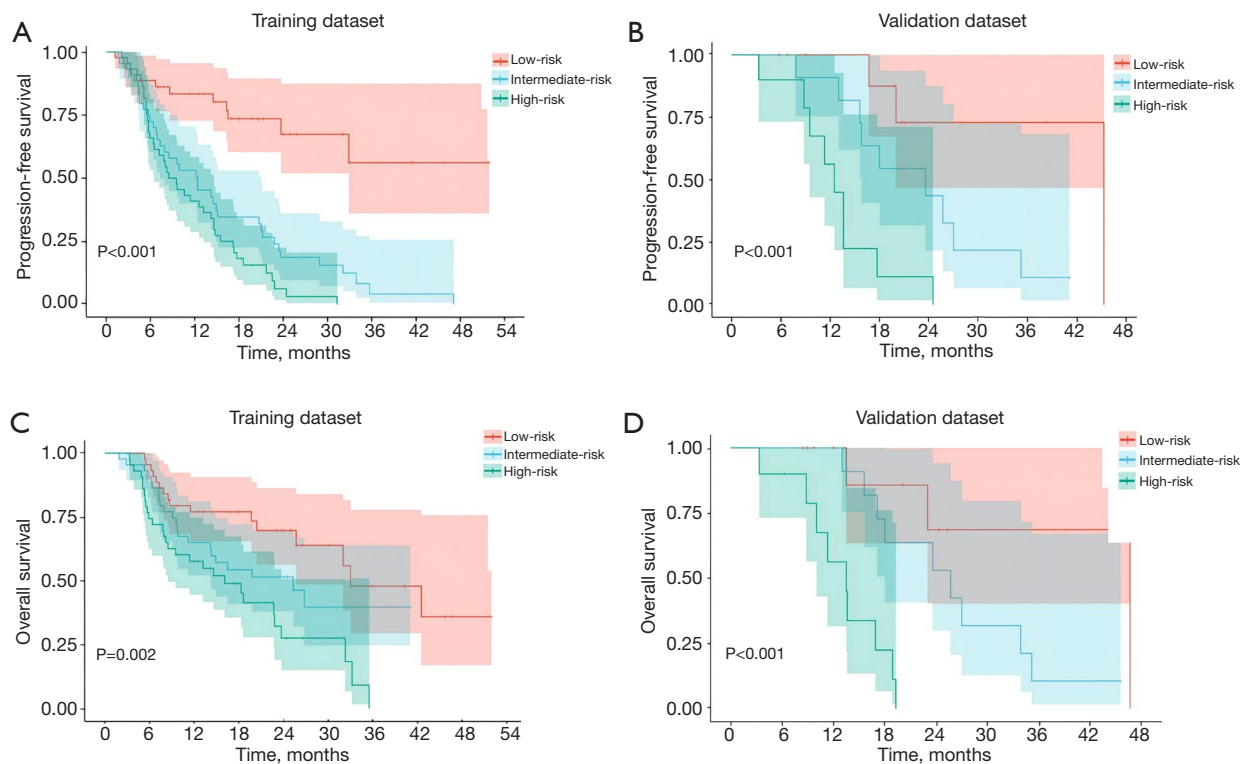


Figure 6 Survival curve analysis of progression-free survival (A,B) and overall survival (C,D) for patients in the high-, intermediate-, and low-risk groups in the training and validation cohorts of the combined model.

score that quantitatively reflects heterogeneity in NSCLC; this score correlated strongly with tumor phenotype and patient survival (39).

Recent investigations underscore subregional radiomics as a novel algorithmic strategy to interrogate tumor heterogeneity via intra-tumoral ecological-diversity metrics (40,41). For instance, Xie *et al.* devised a subregional radiomics model integrating seven distinct features, which accurately predicted OS risk in OSCC patients undergoing definitive CCRT (30). Likewise, magnetic resonance imaging-based quantification of ITH has demonstrated robust performance in forecasting pathological complete response to neoadjuvant chemotherapy in breast cancer. It has exhibited impressive results, with combined AUCs in the training set and three validation sets of 0.90, 0.87, 0.85, and 0.83, respectively, which corroborates our findings (42). The superior prognostic power of subregional radiomics likely stems from its capacity to capture nuanced spatial variations within tumors, distinguishing phenotypic subpopulations more effectively than whole-tumor analyses. Nevertheless, comprehensive analytical assessments are warranted to elucidate the predictive utility of CT-derived intra-tumoral

ecological-diversity characterizations for CCRT efficacy in NSCLC, consistent with recent advances in subregional radiomics (33). We demonstrated a robust association between intra-tumoral ecological-diversity features and CCRT response using multicenter data. Our ITH-prediction model, based solely on these ecological-diversity metrics, outperformed both conventional radiomics and clinical models (AUC =0.78, 0.77). Incorporating clinical and traditional radiomic variables into a combined model further enhanced accuracy (AUC =0.92, 0.87), consistent with prior reports (27,40). Moreover, by applying the ITH model and combined-model threshold, we stratified NSCLC patients receiving CCRT into distinct survival-risk groups: those classified as high-risk experienced significantly shorter PFS and OS. These insights may facilitate early identification of patients unlikely to benefit from CCRT, enabling timely optimization of therapeutic strategies.

Despite its multicenter design, our study has several limitations. First, the absence of external prospective validation and the modest size of our validation cohort may predispose the model to overfitting. Second, selection bias and an imbalanced distribution of clinical stages—

predominantly advanced disease—may undermine the generalizability of our findings. Third, inter-reader variability in tumor delineation can compromise the stability of radiomic features, highlighting the need for robust automated segmentation methods. Finally, the relatively small sample size underscores the imperative for validation in larger, more diverse cohorts to strengthen model reliability.

Conclusions

Our study demonstrated that the pre-treatment contrast-enhanced CT-based ITH model outperformed both conventional radiomic and clinical-feature models in predicting NSCLC patient response to CCRT. A combined model integrating clinical variables, intra-tumoral ecological diversity metrics, and conventional radiomic features further enhanced the accuracy of CCRT outcome prediction in NSCLC. These findings establish a solid foundation for validation in larger, prospective cohorts. Moreover, both the ITH and combined models effectively stratified patients into high- and low-risk groups for PFS and OS, aiding identification of those most likely to benefit from CCRT. Our work provides a novel perspective on forecasting clinical outcomes following CCRT and may facilitate the development of personalized treatment strategies for NSCLC patients.

Acknowledgments

None.

Footnote

Reporting Checklist: The authors have completed the TRIPOD reporting checklist. Available at <https://tcr.amegroups.com/article/view/10.21037/tcr-2025-438/rc>

Data Sharing Statement: Available at <https://tcr.amegroups.com/article/view/10.21037/tcr-2025-438/dss>

Peer Review File: Available at <https://tcr.amegroups.com/article/view/10.21037/tcr-2025-438/prf>

Funding: This work was supported by the Qian Dong Nan Science and Technology Program {grant No. qdnkhJz [2023] 14}, Scientific Research Project of Guizhou Provincial Health and Wellness Commission (grant Nos.

gzwkj2024-099 and gzwkj2025-608), and Spark Program (No. XHJH-0048).

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <https://tcr.amegroups.com/article/view/10.21037/tcr-2025-438/coif>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki and its subsequent amendments. The study was approved by The Second Affiliated Hospital, Guizhou Medical University Ethics Committee (approval No. 2020-LS-03) and individual consent for this retrospective analysis was waived. The other institutions were also informed and approved the study.

Open Access Statement: This is an Open Access article distributed in accordance with the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License (CC BY-NC-ND 4.0), which permits the non-commercial replication and distribution of the article with the strict proviso that no changes or edits are made and the original work is properly cited (including links to both the formal publication through the relevant DOI and the license). See: <https://creativecommons.org/licenses/by-nc-nd/4.0/>.

References

1. Zappa C, Mousa SA. Non-small cell lung cancer: current treatment and future advances. *Transl Lung Cancer Res* 2016;5:288-300.
2. Meza R, Meernik C, Jeon J, et al. Lung cancer incidence trends by gender, race and histology in the United States, 1973-2010. *PLoS One* 2015;10:e0121323.
3. Liang Y, Wakelee HA. Adjuvant chemotherapy of completely resected early stage non-small cell lung cancer (NSCLC). *Transl Lung Cancer Res* 2013;2:403-10.
4. Lococo F, Ghaly G, Flamini S, et al. Artificial intelligence applications in personalizing lung cancer management: state of the art and future perspectives. *J Thorac Dis* 2024;16:7096-110.
5. Remon J, Soria JC, Peters S, et al. Early and locally advanced non-small-cell lung cancer: an update of the ESMO Clinical Practice Guidelines focusing on

- diagnosis, staging, systemic and local therapy. *Ann Oncol* 2021;32:1637-42.
6. Peng J, Zhang X, Hu Y, et al. Deep learning to estimate response of concurrent chemoradiotherapy in non-small-cell lung carcinoma. *J Transl Med* 2024;22:896.
 7. Wu F, Fan J, He Y, et al. Single-cell profiling of tumor heterogeneity and the microenvironment in advanced non-small cell lung cancer. *Nat Commun* 2021;12:2540.
 8. Riely GJ, Wood DE, Ettinger DS, et al. Non-Small Cell Lung Cancer, Version 4.2024, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* 2024;22:249-74.
 9. Pujol JL. Durvalumab Induces Sustained Survival Benefit After Concurrent Chemoradiotherapy in Stage III Non-Small-Cell Lung Cancer. *J Clin Oncol* 2022;40:1271-4.
 10. Duma N, Santana-Davila R, Molina JR. Non-Small Cell Lung Cancer: Epidemiology, Screening, Diagnosis, and Treatment. *Mayo Clin Proc* 2019;94:1623-40.
 11. Zhang Y, Hu Q, Pei Y, et al. A patient-specific lung cancer assembloid model with heterogeneous tumor microenvironments. *Nat Commun* 2024;15:3382.
 12. Marusyk A, Almendro V, Polyak K. Intra-tumour heterogeneity: a looking glass for cancer? *Nat Rev Cancer* 2012;12:323-34.
 13. Kong FM, Ten Haken RK, Schipper MJ, et al. High-dose radiation improved local tumor control and overall survival in patients with inoperable/unresectable non-small-cell lung cancer: long-term results of a radiation dose escalation study. *Int J Radiat Oncol Biol Phys* 2005;63:324-33.
 14. Chen Z, Fillmore CM, Hammerman PS, et al. Non-small-cell lung cancers: a heterogeneous set of diseases. *Nat Rev Cancer* 2014;14:535-46.
 15. Li Q, Wang R, Yang Z, et al. Molecular profiling of human non-small cell lung cancer by single-cell RNA-seq. *Genome Med* 2022;14:87.
 16. Abbosh C, Hodgson D, Doherty GJ, et al. Implementing circulating tumor DNA as a prognostic biomarker in resectable non-small cell lung cancer. *Trends Cancer* 2024;10:643-54.
 17. Nakamura Y, Kawazoe A, Lordick F, et al. Biomarker-targeted therapies for advanced-stage gastric and gastro-oesophageal junction cancers: an emerging paradigm. *Nat Rev Clin Oncol* 2021;18:473-87.
 18. Ilie M, Long-Mira E, Bence C, et al. Comparative study of the PD-L1 status between surgically resected specimens and matched biopsies of NSCLC patients reveal major discordances: a potential issue for anti-PD-L1 therapeutic strategies. *Ann Oncol* 2016;27:147-53.
 19. Dagogo-Jack I, Shaw AT. Tumour heterogeneity and resistance to cancer therapies. *Nat Rev Clin Oncol* 2018;15:81-94.
 20. Su GH, Xiao Y, You C, et al. Radiogenomic-based multiomic analysis reveals imaging intratumor heterogeneity phenotypes and therapeutic targets. *Sci Adv* 2023;9:eadf0837.
 21. Ye G, Wu G, Qi Y, et al. Non-invasive multimodal CT deep learning biomarker to predict pathological complete response of non-small cell lung cancer following neoadjuvant immunochemotherapy: a multicenter study. *J Immunother Cancer* 2024;12:e009348.
 22. Demircioglu A, Grueneisen J, Ingenwerth M, et al. A rapid volume of interest-based approach of radiomics analysis of breast MRI for tumor decoding and phenotyping of breast cancer. *PLoS One* 2020;15:e0234871.
 23. Peng J, Huang J, Huang G, et al. Predicting the Initial Treatment Response to Transarterial Chemoembolization in Intermediate-Stage Hepatocellular Carcinoma by the Integration of Radiomics and Deep Learning. *Front Oncol* 2021;11:730282.
 24. Peng J, Kang S, Ning Z, et al. Residual convolutional neural network for predicting response of transarterial chemoembolization in hepatocellular carcinoma from CT imaging. *Eur Radiol* 2020;30:413-24.
 25. Peng J, Zhang J, Zou D, et al. Deep learning to estimate durable clinical benefit and prognosis from patients with non-small cell lung cancer treated with PD-1/PD-L1 blockade. *Front Immunol* 2022;13:960459.
 26. Perez-Johnston R, Araujo-Filho JA, Connolly JG, et al. CT-based Radiogenomic Analysis of Clinical Stage I Lung Adenocarcinoma with Histopathologic Features and Oncologic Outcomes. *Radiology* 2022;303:664-72.
 27. Chen M, Lu H, Copley SJ, et al. A Novel Radiogenomics Biomarker for Predicting Treatment Response and Pneumotoxicity From Programmed Cell Death Protein or Ligand-1 Inhibition Immunotherapy in NSCLC. *J Thorac Oncol* 2023;18:718-30.
 28. Wang T, She Y, Yang Y, et al. Radiomics for Survival Risk Stratification of Clinical and Pathologic Stage IA Pure-Solid Non-Small Cell Lung Cancer. *Radiology* 2022;302:425-34.
 29. Peng J, Xie B, Ma H, et al. Deep Learning Based on Computed Tomography Predicts Response to Chemoimmunotherapy in Lung Squamous Cell Carcinoma. *Aging Dis* 2024;16:1674-90.
 30. Xie C, Yang P, Zhang X, et al. Sub-region based radiomics

- analysis for survival prediction in oesophageal tumours treated by definitive concurrent chemoradiotherapy. *EBioMedicine* 2019;44:289-97.
31. Zhang Z, Wang Z, Luo T, et al. Computed tomography and radiation dose images-based deep-learning model for predicting radiation pneumonitis in lung cancer patients after radiation therapy. *Radiother Oncol* 2023;182:109581.
 32. Vallières M, Freeman CR, Skamene SR, et al. A radiomics model from joint FDG-PET and MRI texture features for the prediction of lung metastases in soft-tissue sarcomas of the extremities. *Phys Med Biol* 2015;60:5471-96.
 33. Peng J, Zou D, Zhang X, et al. A novel sub-regional radiomics model to predict immunotherapy response in non-small cell lung carcinoma. *J Transl Med* 2024;22:87.
 34. Khouadja O, Naceur MS, Mhamedi S, et al. Tunisian lung cancer dataset: collection, annotation and validation with transfer learning. *International Journal of Advanced Computer Science and Applications* 2024. doi: 10.14569/IJACSA.2024.01507121.
 35. Ang KK, Harris J, Wheeler R, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med* 2010;363:24-35.
 36. She Y, He B, Wang F, et al. Deep learning for predicting major pathological response to neoadjuvant chemoimmunotherapy in non-small cell lung cancer: A multicentre study. *EBioMedicine* 2022;86:104364.
 37. Zhou C, Hou L, Tang X, et al. CT-based radiomics nomogram may predict who can benefit from adaptive radiotherapy in patients with local advanced-NSCLC patients. *Radiother Oncol* 2023;183:109637.
 38. He L, Li Z, Chen X, et al. A radiomics prognostic scoring system for predicting progression-free survival in patients with stage IV non-small cell lung cancer treated with platinum-based chemotherapy. *Chin J Cancer Res* 2021;33:592-605.
 39. Li J, Qiu Z, Zhang C, et al. ITHscore: comprehensive quantification of intra-tumor heterogeneity in NSCLC by multi-scale radiomic features. *Eur Radiol* 2023;33:893-903.
 40. Lüönd F, Tiede S, Christofori G. Breast cancer as an example of tumour heterogeneity and tumour cell plasticity during malignant progression. *Br J Cancer* 2021;125:164-75.
 41. Kim M, Park JE, Kim HS, et al. Spatiotemporal habitats from multiparametric physiologic MRI distinguish tumor progression from treatment-related change in post-treatment glioblastoma. *Eur Radiol* 2021;31:6374-83.
 42. Shi Z, Huang X, Cheng Z, et al. MRI-based Quantification of Intratumoral Heterogeneity for Predicting Treatment Response to Neoadjuvant Chemotherapy in Breast Cancer. *Radiology* 2023;308:e222830. Erratum in: *Radiology* 2023;308:e239021.

Cite this article as: Li B, Huang Z, Hu Y, Du B, Mo Y, He T, Zhao M, Li Y, Zhang M, Xia J, Wang M, Liu X, Lu F, Wang L, Su S, Peng J. Computed tomography-based quantification of intra-tumoral heterogeneity for predicting treatment response to concurrent chemoradiotherapy in non-small cell lung cancer: a multicenter retrospective study. *Transl Cancer Res* 2025;14(9):5504-5519. doi: 10.21037/tcr-2025-438