Original Article

Histopathologic pattern and molecular risk stratification are associated with prognosis in patients with stage IB lung adenocarcinoma

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Background: The benefit of adjuvant therapy remains controversial in completely resected (R0) stage IB non-small cell lung cancer (NCLSC) patients. In this study, we aimed to explore potential prognostic factors in stage IB NSCLC patients.

Methods: This study included 215 patients with R0 stage IB lung adenocarcinoma (LUAD) (tumor size: 3–4 cm). DNA sequencing was performed with surgical samples of 126 patients using a panel of 9 driver genes. The molecular risk stratification was assessed by a 14-gene quantitative polymerase chain reaction assay.

Results: Among the 215 patients, 67.9% had micropapillary/solid (MIP/SOL)-predominant tumors. Epidermal growth factor receptor (*EGFR*) mutations were detected in 75 of 126 patients (59.5%). MIP/SOL tumors harbored less common *EGFR* mutations than the other histologic patterns (50.6% *vs.* 79.5%, P=0.003). Molecular risk stratification was successfully assessed in 99 patients, of whom 37.4%, 26.3%, and 36.4% were high, intermediate, and low risk, respectively. The MIP/SOL pattern was associated with shorter disease-free survival (DFS) [hazard ratio (HR) =2.16, 95% confidence interval: 1.28–3.67; P=0.01]. The molecular high-risk patients had shorter DFS than the low- (HR =2.93, P=0.01) and intermediate-risk patients (HR =2.35, P=0.06). The prognostic value of molecular risk stratification was also significant in the MIP/SOL subset (median DFS high-risk: 45 months, low and intermediate risk: not reached; P=0.03).

Conclusions: Our study showed that both the MIP/SOL pattern and molecular high-risk category were adverse prognostic factors in stage IB NSCLC patients. Our results suggest that combining histologic classification and molecular risk stratification may help to identify the subset of patients with poor prognosis.

Keywords: Stage IB lung adenocarcinoma (stage IB LUAD); disease-free survival (DFS); micropapillary/solid pattern (MIP/SOL pattern); molecular risk stratification; prognostic factor

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Introduction

Lung cancer remains the leading cause of cancer-related death worldwide and in China (1). Non-small cell lung cancer (NSCLC) accounts for 70–80% of all lung cancer cases (2). With the ongoing adoption of lung cancer screening (3), the number of patients diagnosed with early-stage disease is increasing. Despite having the opportunity for radical surgery, 35–50% of patients with early-stage NSCLC relapse after resection (4).

Adjuvant chemotherapy is one of the standard-of-care therapies for completely resected (R0) stage II–III NSCLC. However, the benefit of adjuvant therapy remains controversial in patients with R0 stage IB NSCLC (5-11). Notably, up to 30% of stage IB patients develop recurrence within 5 years post-surgery (12,13). Currently, the National Comprehensive Cancer Network (NCCN) guidelines recommend adjuvant chemotherapy for stage IB and stage IIA patients who have high-risk factors including poorly differentiated tumors, tumors >4 cm in size, vascular invasion, wedge resection, visceral pleural involvement, and unknown lymph node status. However, these factors should not serve as independent indicators (14). Thus, patients with stage IB NSCLC need to be more accurately stratified to select those who may benefit from adjuvant systemic therapy. Prognostic markers are helpful to guide adjuvant therapy if they can identify patients with potential poor prognosis for stage IB NSCLC patients.

Highlight box

Key findings

- Both the micropapillary/solid (MIP/SOL) pattern and molecular high-risk category are adverse prognostic factors in stage IB lung adenocarcinoma (LUAD) patients with a tumor size ≤4 cm.

What is known, and what is new?

- The MIP/SOL pattern is more aggressive than other histologic patterns in LUAD. Molecular high-risk LUAD patients have shorter disease-free survival than low- and intermediate-risk patients.
- The MIP/SOL tumors harbored less common epidermal growth factor receptor (*EGFR*) mutations than the other histologic patterns, and the prognostic value of molecular risk stratification was also significant in the MIP/SOL subset.

What is the implication, and what should change now?

- Combining the histologic pattern and molecular risk stratification may help to identify the subset of stage IB LUAD patients with poor prognosis.

The International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society (IASLC/ATS/ERS) classify invasive lung adenocarcinoma into the following five subtypes based on their predominant histologic pattern: lepidic (LEP), papillary (PAP), acinar (ACN), micropapillary (MIP), and solid (SOL). Studies have demonstrated that patients with the MIP or SOL subtype have the worst prognosis and benefit from adjuvant chemotherapy (15-18). It has also been reported that the prognostic benefit of adjuvant chemotherapy differs based on the epidermal growth factor receptor (*EGFR*) mutation status of patients with stage IB–IIIA primary lung adenocarcinoma (19,20). Moreover, a 14-gene expression assay (DetermaRx) has been developed that has been shown to have prognostic and predictive value in patients with resected early-stage non-squamous non-small cell lung cancer (21-23). Stage IA–IIA patients identified as high/intermediate risk by this molecular assay had a more beneficial 5-year disease-free survival (DFS) rate when treated with adjuvant platinum chemotherapy than those not treated with adjuvant chemotherapy (91.7% *vs.* 48.9%, P=0.004) (24), regardless of their *EGFR* status (23). However, whether the above factors can be used for prognostic stratification in stage IB patients has not been studied.

Based on the above-mentioned evidence, we sought to explore the prognostic significance of the histologic subtype, *EGFR* status, and molecular risk stratification in R0 patients with stage IB LUAD. Our findings may help identify candidates suitable for adjuvant therapy. We present this article in accordance with the REMARK reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-506/rc).

Methods

Patients and study design

We retrospectively enrolled patients with pathologic stage IB LUAD who underwent surgery at the Tianjin Medical University Cancer Institute and Hospital between April, 2014 and October, 2018. To be eligible for inclusion in this study, the patients had to meet the following inclusion criteria: (I) be aged ≥18 years; (II) be systemic treatment naïve; (III) have undergone a pathological assessment of a resected specimen that confirmed stage IB disease according to the staging criteria of the American Joint Committee on Cancer (AJCC) 8th edition; (IV) have one

of the five adenocarcinoma histologic subtypes confirmed pathologically; (V) have undergone radical R0 resection; and (VI) have a tumor 3–4 cm in size.

Surgical samples of patients were sequenced using a panel that included 9 lung cancer driver genes. The molecular risk stratification was assessed by a 14-gene quantitative polymerase chain reaction (qPCR) assay (DetermaRxTM, Burning Rock Biotech, Guangzhou, China). Data on the patients' clinical characteristics, treatment information, and DFS were collected. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the ethics committee of Tianjin Medical University Cancer Institute and Hospital (No. Ek2021272). The requirement of informed consent was waived because of the retrospective and observational nature of the study.

DNA sequencing

Genomic DNA was extracted from the tissue samples using the QIAamp DNA FFPE tissue kit (Qiagen, Hilden, Germany) per the manufacturer's instructions. Capture-based targeted sequencing was performed using a 9-gene panel (OncoScreen® Focus CDx Tissue Kit, Burning Rock Biotech) as previously described (25-27). The panel covers the 9 classical driver genes in lung cancer (i.e., *EGFR*, *MET*, *ERBB2*, *KRAS*, *BRAF*, *PIK3CA*, *ALK*, *ROS1*, and *RET*). Data analyses, including variant calling and interpretation, and copy number variation analyses, were performed using optimized pipelines based on the methods described previously (27).

Risk stratification by the 14-gene assay

Total RNA was extracted using the RNeasy FFPE Kit (Qiagen, Hilden, Germany) per the manufacturer's instructions. A nanodrop spectrometer (Thermo Scientific, Wilmington, DE, USA) was used to evaluate the extraction yield (ng) and purity (A260/280 and A260/230 absorbance ratios) of the extracted RNA samples. The extracted RNA was subjected to reverse transcription, complementary DNA synthesis, and TaqMan qPCR amplification as described previously (21). Briefly, the expression of 11 cancer-related target genes (i.e., *BAG1*, *BRCA1*, *CDC6*, *CDK2AP1*, *ERBB3*, *FUT3*, *IL11*, *LCK*, *RND3*, *SH3BGR*, and *WNT3A*) and three housekeeping genes (i.e., *ESD*, *TBP*, and *YAP1*) were quantified. The comparative cycle threshold (ct) method was used to assess the relative expression of each target gene. A continuous risk score was generated for each individual using the model previously developed based on the relative expression values of the 11 targeted genes. Patients were categorized as low-, intermediate-, and high-risk according to their risk scores using the established cut-off values (21).

Statistical analysis

All the statistical analyses were performed using GraphPad Prism (version 8.4.3, Boston, MA, USA). Differences between groups were compared using the chi-squared test or Fisher's exact test for the categorical variables. A Kaplan-Meier analysis was used to estimate survival, and a log-rank test was used to determine the differences in the multiple survival metrics between the groups. The hazard ratio (HR) and associated 95% confidence interval (CI) were calculated using a Cox proportional hazards model. Statistical significance was defined as a P value <0.05.

Results

Baseline characteristics

A total of 215 patients with R0 stage IB LUAD were included in the study. As summarized in *Table 1*, the median age of the cohort was 62 years (range, 36–80 years). Among these patients, 117 of 215 patients (54.4%) were female and 121 of 215 patients (56.3%) had no smoking history. Of the 215 patients, 146 (67.9%) had tumors with the MIP/SOL-predominant pattern. In total, 87 (40.5%) patients received platinum-based doublet adjuvant chemotherapy and 11 (5.1%) received adjuvant targeted therapy with or without chemotherapy. At a median follow-up time of 59 months, 61 (28.4%) patients had experienced recurrence. The median DFS of the recurrent patients was 24 months.

Genomic and molecular characteristics between histologic subtypes

Samples from 126 patients underwent DNA sequencing and generated eligible data. *EGFR* mutations were detected in 75 of the 126 patients (59.5%), including 19 del (n=31), L858R (n=32), and uncommon variants (n=12) (*Figure 1A*). The *EGFR* mutations occurred less commonly in the tumors with the MIP/SOL pattern than those with other histologic patterns (50.6% *vs.* 79.5%, P=0.003, *Figure 1B*). Moreover, alterations were also detected in *KRAS* (11%),

Table 1 Patient characteristics

Characteristic	Values (n=215)
Age, median [range], years	62 [36–80]
Sex, n (%)	
Female	117 (54.4)
Male	98 (45.6)
Smoking history, n (%)	
Yes	93 (43.3)
No	121 (56.3)
Unknown	1 (0.5)
Histologic subtype, n (%)	
Solid/micropapillary	146 (67.9)
Lepidic/papillary/acinar	69 (32.1)
Adjuvant treatment, n (%)	
No	117 (54.4)
Chemotherapy	87 (40.5)
Targeted ± chemotherapy	11 (5.1)
Median follow-up, months (95% CI)	59 (57.0–60.0)
Median DFS in recurrent patients, months (95% CI)	24 (17.0–29.0)

CI, confidence interval; DFS, disease-free survival.

MET (6%), *ERBB2* (5%), *ROS1* (5%), *PIK3CA* (4%), *ALK* (3%), and *RET* (2%). Molecular risk stratification based on the 14-gene assay was successfully assessed in 99 patients, of whom 37.4%, 26.3%, and 36.4% were predicted to be high-, intermediate-, and low-risk, respectively, as described in the "Methods" section. We observed a higher high-risk proportion (46.2% *vs.* 20.6%) and a lower low-risk proportion (26.2% *vs.* 55.9%) in the MIP/SOL pattern than the LEP/PAP/ACN patterns (P=0.009, *Figure 1C*).

Association between survival and genomic, molecular and histologic characteristics

The results of the survival analysis revealed that the patients who had received adjuvant therapy had comparable DFS to those who did not receive adjuvant therapy (P=0.38, *Figure 2A*), which suggests the need for further stratification of patients with stage IB disease. We first investigated the prognostic role of the histologic patterns. The patients with the MIP/SOL pattern had significantly inferior DFS than those with other histologic patterns (HR: 2.16, 95% CI: 1.28–3.67; P=0.01, *Figure 2B*). However, a further stratification analysis failed to identify the DFS benefit of adjuvant therapy in stage IB patients irrespective of the tumor histologic patterns (*Figure 2C*,*2D*).

We also examined the role of *EGFR* mutations in predicting patient prognosis. There was no difference in DFS between patients with or without EGFR mutation in total patients or stratified by MIP/SOL subtypes (P=0.22, 0.70, 0.99, respectively, *Figure 3*). There was also no difference in DFS between patients with EGFR 19del, L858R or other EGFR mutations in total patients or stratified by MIP/SOL subtypes (P=0.50, 0.58, 030, respectively, Figure S1). Moreover, *EGFR* status was not a predictive marker for adjuvant chemotherapy, as patients treated with adjuvant chemotherapy did not achieve beneficial DFS compared with those who were not treated with adjuvant therapy, irrespective of their *EGFR* status (Figure S2). The *EGFR*-mutant patients appeared to achieve a survival benefit if they received adjuvant targeted treatment; however, the sample size was too small to observe a significant difference (Figure S2B).

The patients who were stratified as high risk by the 14-gene assay had shorter DFS than those who were stratified as low (HR =2.93, P=0.01) and intermediate risk (HR =2.35, P=0.06) (*Figure 4A*). The patients who were high risk had shorter DFS than those with low/intermediate risk (HR =2.68, P=0.005, *Figure 4B*). Interestingly, the prognostic significance of the molecular risk stratification was only seen in the subset of patients with the MIP/SOL pattern (median DFS high risk: 45 months, low and intermediate risk: NR, P=0.03, *Figure 4C*), but not the other histologic subtypes (P=0.26, *Figure 4D*).

The efficacy of adjuvant therapy in high-risk patients was preliminarily investigated in this study. Among the 37 high-risk LUAD patients, 16 patients received adjuvant therapy (1 with icotinib plus pemetrexed and 15 with platinum-based doublet chemotherapy), while the remaining patients did not receive adjuvant therapy. Survival analysis revealed a comparable DFS between high-risk patients with and without adjuvant therapy (Figure S3A, P=0.63). In the subgroup analysis of 30 MIP/SOL LUAD patients classified as high-risk, 14 patients received adjuvant therapy (1 with icotinib plus pemetrexed and 13 with platinum-based doublet chemotherapy) and the remaining patients did not receive adjuvant therapy. Similarly, a comparable DFS was also observed between patients with and without adjuvant therapy (Figure S3B, P=0.89).

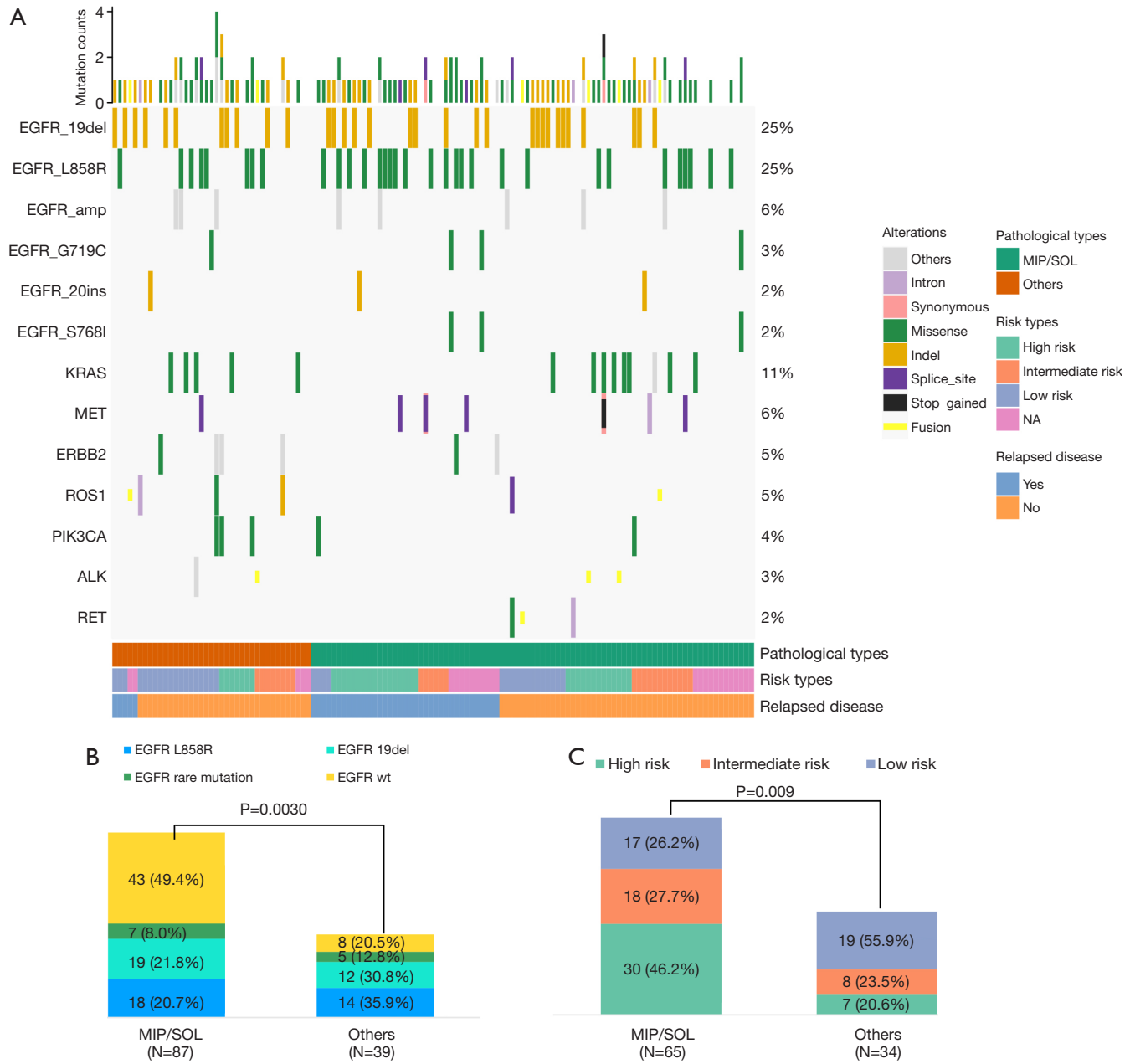

Figure 1 Differential genomic and molecular characteristics between histologic subtypes. (A) Oncoprint showing the landscape of genomic alterations; (B) *EGFR* mutations in different histologic subtypes; (C) molecular risk groups in different histologic subtypes. EGFR, epidermal growth factor receptor; MIP/SOL, micropapillary/solid.

Multivariable analysis of the association between survival and histological subtypes and molecular risk

We then explored whether the MIP/SOL pattern and molecular risk were independent risk factors for prognosis. In the multivariable Cox proportional hazards regression model that included sex, age, smoking history, histological subtype, and molecular risk stratification, both the histological subtype (HR: 3.49, 95% CI: 1.21–10.08, P=0.02) and molecular risk stratification (HR: 2.79, 95% CI: 1.25–6.20, P=0.01) retained a significant association with DFS (*Table 2*). Our data suggest that the histological

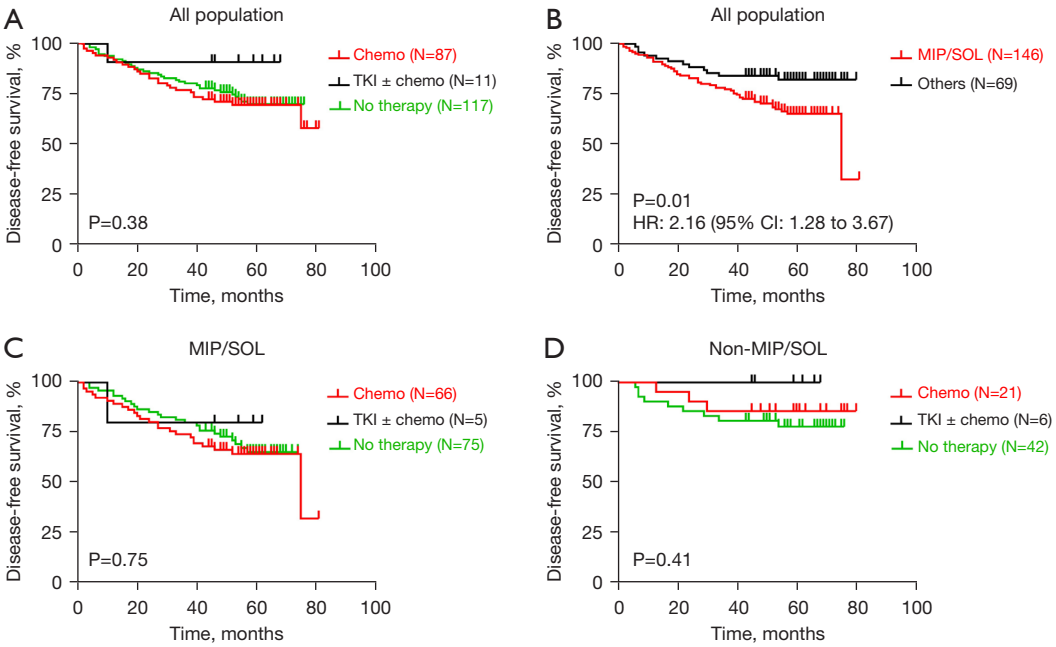

Figure 2 Comparison of DFS among patients with different types of histology and patients treated with different types of adjuvant therapy. (A) DFS among patients with different types of adjuvant therapy; (B) DFS between patients with the MIP/SOL histologic type and other histologic types; (C) DFS among the MIP/SOL patients with different types of adjuvant therapy; (D) DFS among the non-MIP/SOL patients with different types of adjuvant therapy. DFS, disease-free survival; MIP/SOL, micropapillary/solid; HR, hazard ratio; CI, confidence interval; TKI, tyrosine kinase inhibitor.

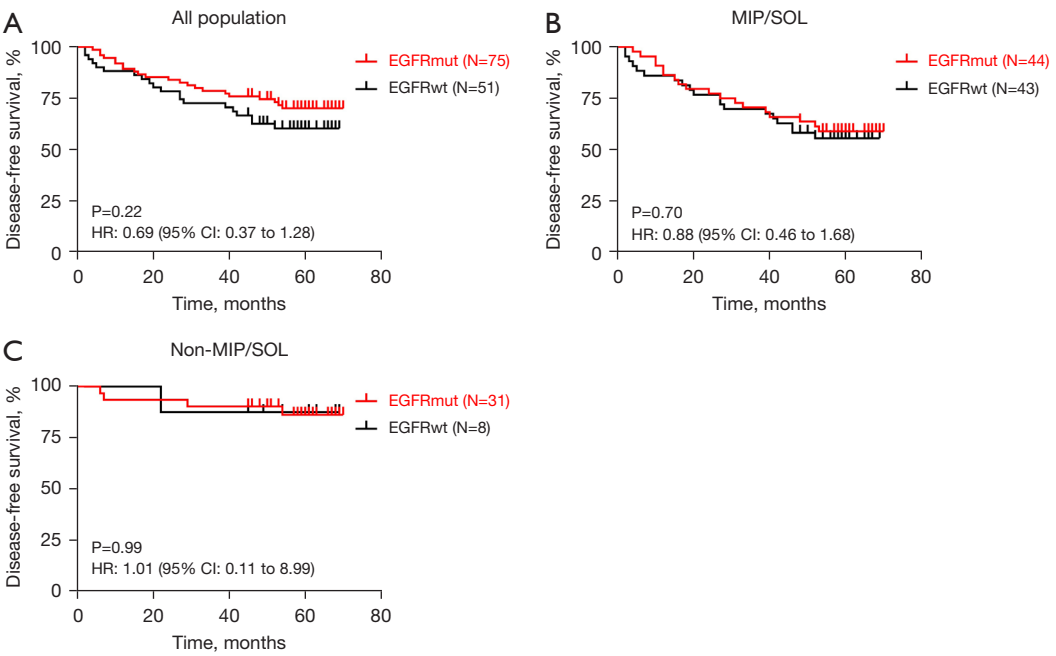

Figure 3 Comparison of DFS among patients with different *EGFR* mutation status types. (A) In all patients; (B) in patients with the MIP/SOL histology; (C) in patients with the non-MIP/SOL histology. DFS, disease-free survival; EGFR, epidermal growth factor receptor; MIP/SOL, micropapillary/solid; HR, hazard ratio; CI, confidence interval.

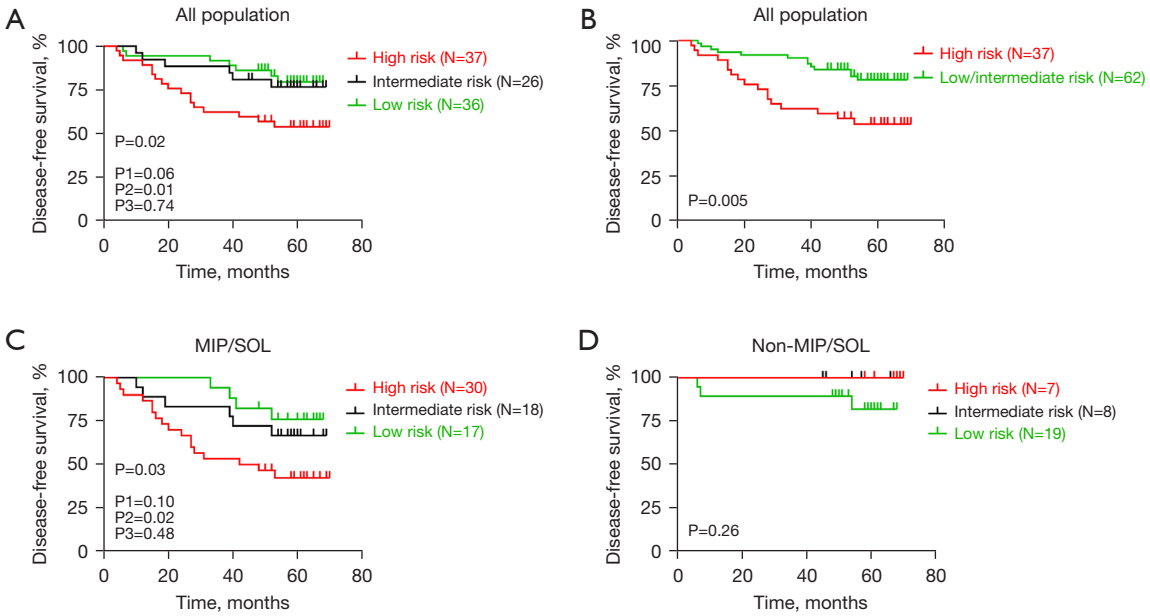

Figure 4 Stratification of patients by histology and molecular risk score. (A) The association of the molecular risk with DFS in all patients; (B) the comparison of DFS between high risk and low/intermediate risk in all patients; (C) the association of the molecular risk with DFS in patients with the MIP/SOL histology; (D) the association of the molecular risk with DFS in patients with the non-MIP/SOL histology. P1, P2, and P3 indicated the P value of the comparison between the high- and intermediate-risk group, between the high- and low-risk groups, and between the intermediate- and low-risk groups, respectively. DFS, disease-free survival; MIP/SOL, micropapillary/solid.

Table 2 Multivariable analysis of risk factors of DFS

Characteristic	Multivariable analysis	
	HR (95% CI)	P value
Sex (male *vs.* female)	0.99 (0.36–2.71)	0.99
Age (≥60 *vs.* <60 years)	0.72 (0.34–1.53)	0.39
Smoking history (with *vs.* without)	0.62 (0.21–1.81)	0.38
Histological subtype of tumor (MIP/SOL *vs.* others)	3.49 (1.21–10.08)	0.02
Molecular risk stratification (high-risk patients *vs.* others)	2.79 (1.25–6.20)	0.01

DFS, disease-free survival; MIP/SOL, micropapillary/solid; HR, hazard ratio; CI, confidence interval.

subtype and molecular risk stratification are independent risk factors for prognosis. Moreover, the subset of patients who had a MIP/SOL tumor molecularly categorized as high risk had the worst prognoses.

Discussion

Adjuvant therapy, including chemotherapy alone or plus tyrosine kinase inhibitors (TKIs)/immune checkpoint inhibitors (ICIs), is currently recommended for patients with resectable stage II–III NSCLC; however, its benefit in patients with stage IB NSCLC remains controversial. Several studies have shown that stage IB NSCLC patients did not achieve any survival benefits from adjuvant chemotherapy (7,28,29). Conversely, stratification analyses of the CALGB 9633 and JBR-10 trials found that stage IB patients with a tumor size >4 cm tended to achieve survival benefits (5,7). In another retrospective study, stage IB patients with tumors >3.2 cm in size benefited from platinum-based adjuvant chemotherapy (30). Despite the approval of osimertinib for the adjuvant treatment of stage IB *EGFR*-mutant patients, the subgroup analysis of the ADAURA study demonstrated that these patients benefit less from osimertinib than those with stage II or III disease (31). Notably, the Food and Drug Administration approved atezolizumab for adjuvant treatment only for stage II–IIIA patients with programmed cell death ligand-1 (PD-L1) positive NSCLC based on the results of the IMpower010 study (32). The KEYNOTE-091 study led to the approval of pembrolizumab for the treatment of stage IB (T2a ≥4 cm), II, or IIIA NSCLC (33). Thus, it appears to be clinically crucial to identify the stage

IB patients who may benefit from adjuvant treatment, especially for those with a tumor size ≤4 cm.

In our study, we enrolled R0 stage IB patients with tumors 3–4 cm in size, and found that adjuvant therapy did not result in better DFS in this cohort. Similarly, a retrospective study of 1,005 patients with node-negative stage IB tumors found that adjuvant chemotherapy was not associated with DFS or OS, regardless of the tumor size (34). Conversely, two retrospective studies based on data from the National Cancer Data Base (NCDB) showed that patients with R0 stage IB tumors measuring <4 cm also benefited from adjuvant chemotherapy (35,36). These heterogeneous results indicate the potential factors that may affect the prognosis of patients with R0 stage IB disease.

Interestingly, the MIP/SOL pattern and molecular high-risk categorization were identified as predictive factors for shorter DFS in R0 stage IB patients with tumors measuring 3–4 cm. The histologic MIP/SOL pattern has been associated with a higher possibility of recurrence and shorter OS in resected lung adenocarcinoma compared with the histologic LEP/PAP/ACN patterns (15,17). Another study revealed that patients with the MIP/SOL pattern had shorter DFS but comparable OS to those with the LEP/ACN/PAP patterns (P<0.01). Only the MIP/SOL subgroup obtained DFS benefits from adjuvant chemotherapy (HR: 0.60; 95% CI: 0.44–0.82; interaction P<0.01) (16). Similarly, our study demonstrated for the first time the prognostic value of the MIP/SOL pattern for inferior DFS in the subset of stage IB patients with tumors <4 cm in size. Conversely, the MIP/SOL pattern did not predict a DFS benefit in the patients who received adjuvant chemotherapy in our cohort.

The 14-gene-based molecular prognostic classifier has been developed and validated in several studies. The 5-year OS was 71.4% (95% CI: 60.5–80.0%), 58.3% (95% CI: 48.9–66.6%), and 49.2% (95% CI: 42.2–55.8%) in the low-, intermediate-, and high-risk patients with stage I non-squamous NSCLC (nsqNSCLC), respectively (P_{trend}<0.001). Similarly, in patients with stage I–III disease, the 5-year OS was 74.1% (95% CI: 66.0–80.6%), 57.4% (95% CI: 48.3–65.5%), and 44.6% (95% CI: 40.2–48.9%) in the high-, intermediate-, and low-risk groups, respectively (P_{trend}<0.001) (21). Another validation study of node-negative stage T1a patients showed that 5-year OS differed significantly among the following molecular groups: high-risk (52.3%; 95% CI: 41.1–62.4%), intermediate-risk (69.1%; 95% CI: 56.8–78.6%), and low risk (83.0%; 95% CI: 72.8–89.7%) (22). Moreover, a retrospective study on stage IA, IB, and IIA nsqNSCLC reported comparable 5-year DFS between the low- (93.8%) and high-/intermediate-risk patients treated with adjuvant chemotherapy (91.7%), which was higher than that of the high-/intermediate-risk patients who did not receive adjuvant chemotherapy (48.9%, P=0.004) (24). A recent prospective study also found adjuvant chemotherapy prolonged survival in molecular high-/intermediate-risk patients with stage IA–IIA [5-year freedom from recurrence (FFR): 97.0% *vs.* 72.4%, log-rank P value <0.001] and stage IA disease (5-year FFR: 100% *vs.* 73.2%, log-rank P value <0.001) (23). We performed the first study to investigate the prognostic and predictive value of the molecular classifier in stage IB patients with a tumor size <4 cm. Our results confirmed its prognostic role in this specific cohort of patients. Integrating both the histologic and molecular classification revealed a subset of patients with the worst DFS who had MIP/SOL-predominant and molecular high-risk tumors.

Our study also found that *EGFR* mutation status was not associated with DFS or the survival benefit of adjuvant chemotherapy. This supports the results of Woodard *et al.*, who also found that molecular risk stratification is independent of *EGFR* status in patients with stage IA–IIA disease (23). In our study, we observed that the *EGFR*-mutant patients showed a trend of better DFS when treated with TKIs than other adjuvant therapies (Figure S2B); however, this difference was not significant due to the sample size and short follow-up period. Therefore, treatment decision-making combining molecular risk stratification and histologic classification with *EGFR* mutation status may better inform adjuvant therapy recommendations.

Comparable survival was observed between patients with and without adjuvant therapy in high-risk group and high-risk MIP/SOL group. This finding may be attributed to the relatively small sample size, as only about 30 high-risk patients were included in this work. Conducting a large, prospective cohort study is warranted to further explore the effectiveness of adjuvant therapy in high-risk patients and investigate the optimal adjuvant regimens for this patient population.

Our study had several limitations. First, the follow-up period was short, and the median DFS was not reached. Second, since the enrolled patients received treatment between 2014 and 2018 when the EGFR TKI had not been approved for adjuvant use in NSCLC, most *EGFR*-mutant patients received chemotherapy alone. This might have affected our ability to observe the predictive value of the

EGFR mutation for TKIs. Thirdly, the subgroup analyses of the survival curves with small sample sizes were not convincing. An independent validation cohort with larger sample sizes to verify the findings in the study would be appreciated in the future. Forth, the interpretation of the results needs to be cautious due to the retrospective nature of the study and potential selection bias.

Conclusions

In conclusion, our study confirmed that the MIP/SOL pattern is an adverse prognostic factor for DFS in stage IB LUAD patients with a tumor size ≤4 cm. Moreover, the qPCR-based assay was able to reliably identify MIP/SOL patients with a worse prognosis.

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Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-506/rc

Data Sharing Statement: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-506/dss

Peer Review File: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-506/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-506/coif). L.S., H.D. and S.C. are employees of Burning Rock Biotech, Guangzhou, China. The other 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 (as revised in 2013). The study was approved by the ethics committee of Tianjin Medical University Cancer Institute and Hospital (No. Ek2021272). The requirement of informed consent was waived because of the retrospective and observational nature of the study.

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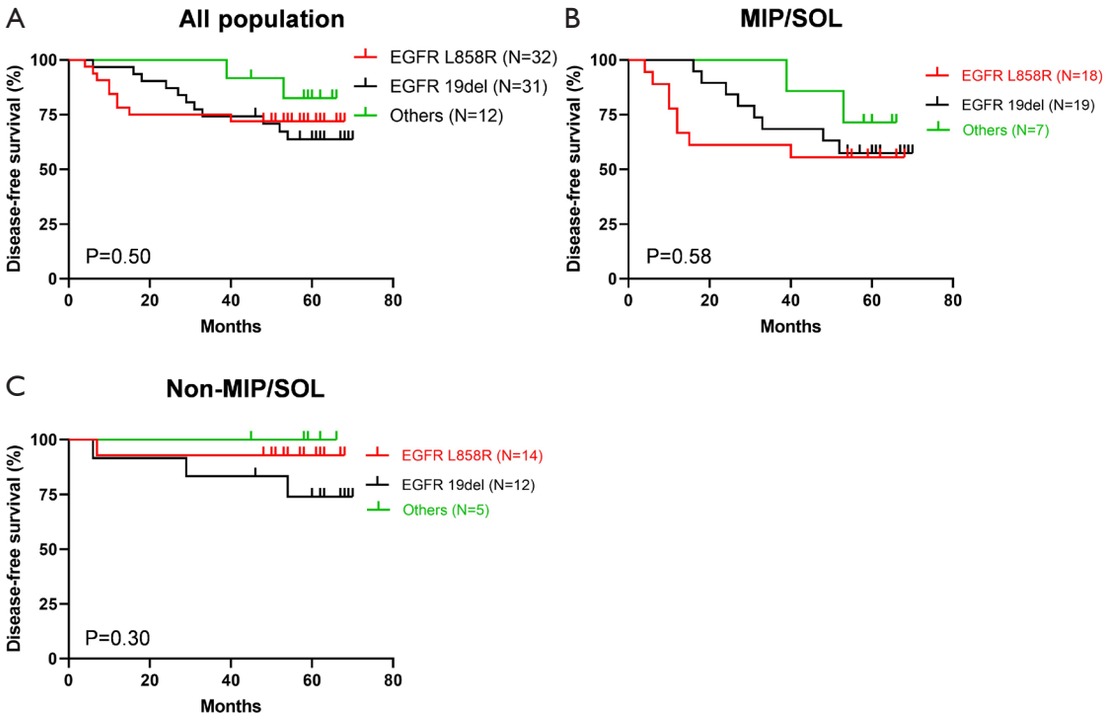

Figure S1 The association between the *EGFR* mutation type and DFS. (A) DFS among patients with different EGFR mutations; (B) DFS among the MIP/SOL patients with different EGFR mutations; (C) DFS among the non-MIP/SOL patients with different EGFR mutations. DFS, disease-free survival; EGFR, epidermal growth factor receptor; MIP/SOL, micropapillary/solid.

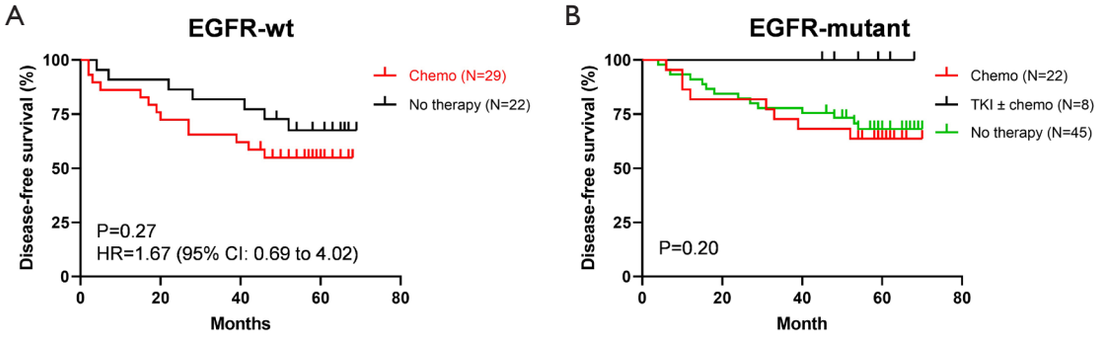

Figure S2 The association between adjuvant therapy and DFS in the patients with different EGFR status types. *EGFR*, epidermal growth factor receptor; DFS, disease-free survival; TKI, tyrosine kinase inhibitor.

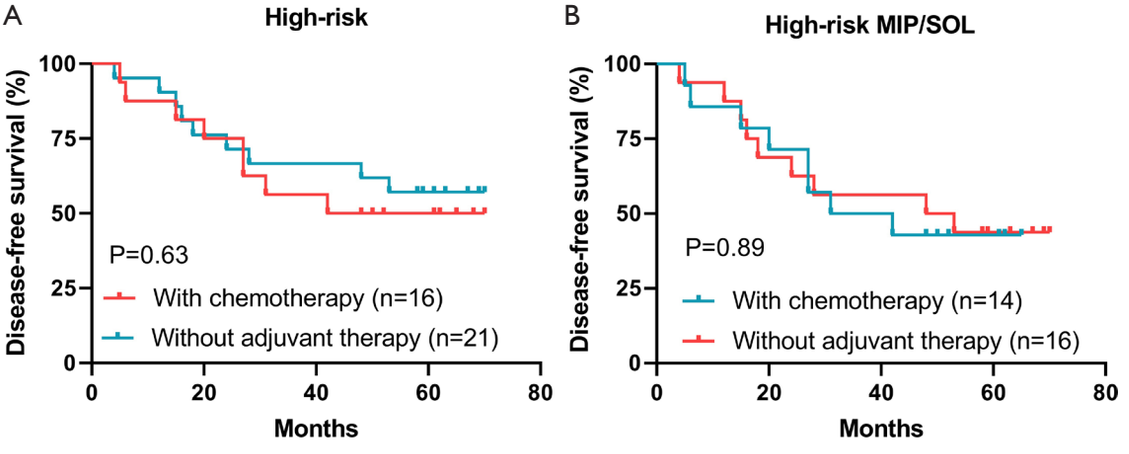

Figure S3 The survival difference between patients with and without adjuvant therapy in high-risk (A) and high-risk MIP/SOL group (B). DFS, disease-free survival; MIP/SOL, micropapillary/solid.

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