Original Article

Real-world biomarker testing patterns: clinical-pathological portrait of early and late non-small cell lung cancer in hub and spoke North Italian centers

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Background: Lung cancer is still the main cause of cancer death. In the last decades, significant innovations were introduced in non-small cell lung cancer (NSCLC) treatment and management improving patient outcomes. The discovery of immune checkpoint inhibitors and the detection of an increasing list of actionable genetic alterations are enabling a tailored approach. Herein, we assessed in a pragmatic retrospective study the rate of biomarker tests within a large pulmonary pathology-based unit (PPU) network of the Veneto region (Northern Italy).

Methods: Each PPU of 7 hubs and spoke centers implemented a biomarker database with pathologic and clinical data of patients with NSCLC diagnosis over 24 months.

Results: Out of 1,817 NSCLC cases, 51% were advanced and 49% early stage, with 72% being adenocarcinomas. Programmed death ligand 1 expression and epidermal growth factor receptor mutations were available in most samples, 91% and 78%, respectively. Only 36% of advanced stages received all 5 biomarker tests with an increased rate over time. Co-occurring molecular alterations were detected in 42 cases (2%): the prevalence was (n=17) 41% and (n=25) 59% in early and late-stage adenocarcinomas, respectively.

Conclusions: In this real-world study, while most patients received at least one biomarker test, less than 50% had all 5 biomarkers. The screening appeared to increase over time especially with the progressive use of next generation sequencing. Our results confirm the importance of systematic biomarker testing including all NSCLCs based on the evidence of several genomic alterations also in early-stage disease whose analysis may become relevant as neo-adjuvant targeted therapies are available.

Keywords: Non-small cell lung cancer (NSCLC); biomarkers; actionable targets; lung cancer

Submitted Jan 30, 2024. Accepted for publication Apr 29, 2024. Published online Jul 25, 2024.
doi: 10.21037/tlcr-24-107
View this article at: https://dx.doi.org/10.21037/tlcr-24-107

Introduction

Lung cancer is the first cause of cancer-related death worldwide. Most cases are diagnosed in the advanced stage when tumor-related symptoms become evident (1). The revolutionary progresses in lung cancer screening, diagnosis, and treatment will hopefully modify this scenario in the near future. Among these, the use of powerful treatment options relying on the detection of molecular and immune biomarkers has had an important impact on non-small cell lung cancer (NSCLC) patient survival (2). Current guidelines (3-5) strongly recommend testing for actionable targets including epidermal growth factor receptor (*EGFR*), proto-oncogene B-Raf (*BRAF*) V600, anaplastic lymphoma kinase (*ALK*), c-ros oncogene 1 (*ROS1*), *MET* exon 14 skipping mutations and amplifications; *RET* rearrangements, Kirsten Rat Sarcoma viral oncogene (*KRAS*) G12C, human epidermal growth factor 2 receptor (*HER2*) mutations, neurotrophin receptor tyrosine kinase (*NTRK1–3*) and programmed death ligand 1 (PD-L1) in tumor samples from patients with metastatic NSCLC to support treatment decision making. Based on recent evidence (6-8) to select patients for adjuvant treatment, the international guidelines have been updated with the expansion of routine molecular analysis to all early-stage lung adenocarcinomas (3,9). Since 2017, guidelines have also reported next-generation sequencing (NGS) testing, at least using limited gene panel testing, has been recognized as an option for multiplex biomarker testing (10). NGS can detect a broad spectrum of alterations, including not only emerging actionable mutations but also concomitant mutations that may be responsible for targeted therapy resistance (11,12). However, upfront NGS testing of advanced NSCLC compared to sequential panel evaluation is still a matter of debate with a major issue being lab expertise and overall cost of the procedure (13,14).

Veneto was one of the first regions in Italy to share a lung cancer care pathway among hub (3 centers) and spoke hospitals (4 centers). Inspired by the European Cancer Organization essential requirements for quality cancer care (15), it draws the lines to identify precise diagnostic paths between different specialists, strengthening collaboration of hub structures and most of the spoke centers in Veneto (16). The systematic assessment of biomarkers in advanced NSCLC—and, based on new evidence, even in early stages (6-8)—and the weekly multidisciplinary discussions along with the annual updated scientific meetings between the pulmonologist and pathologist unit (PPU), can be considered the milestones of this document. Although several short-term performance indicators are included in our lung cancer care pathway, a systematic analysis of effective adherence to biomarker investigation coming from a large PPU network experience has never been reported. Thus, in the present study we sought to explore the current real-world diagnostic journey mainly focusing on molecular assessment and its performance in all Veneto hub and spoke centers.

Highlight box

Key findings
- The assessment of most biomarkers is still challenging in advanced non-small cell lung cancer (NSCLC).
- Hub can support spoke centers by providing complementary techniques.
- The co-occurrence of gene alterations highlights a deep molecular assessment.
- Immune and molecular changes are similarly altered in early and advanced NSCLC.
- Clinical/pathological/molecular correlations can improve prognostic/predictive grounds.

What is known and what is new?
- The immune/molecular signature is key in NSCLC patients' management
- We described the extent and effectiveness of pathologic and clinical characterization in NSCLC in a real-world context, highlighting the importance of hub and spoke cooperation.

What is the implication, and what should change now?
- The complexity of NSCLC immune/molecular profile requires deep and complete understanding.
- Our data foster the collaboration between clinicians and pathologists and support the interaction between hub and spoke centers to achieve the best management of NSCLC patients.

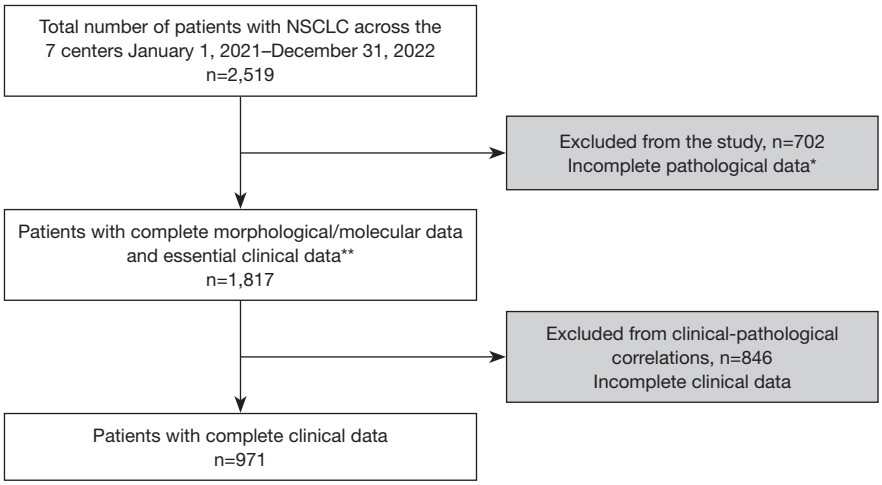

Figure 1 Flow chart of study population selection. The gray boxes show the subjects excluded from the study (see exclusion criteria). *, incomplete pathological data: no clear-cut definition of the histology type (usually due to inadequate samples); **, essential clinical data: gender, age, major symptoms, diagnostic procedure, tumor staging. NSCLC, non-small cell lung cancer.

Correlations between molecular and clinical phenotypes have also been investigated. Furthermore, the study summarizes the experience shared among different PPUs in Veneto during annual meetings held over the last two years. We present this article in accordance with the STROBE reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-107/rc).

Methods

Study design

Using a multicenter retrospective observational study, we examined demographic and clinical characteristics, and biomarker testing patterns of patients diagnosed with NSCLC between January 1, 2021, and December 31, 2022, across 7 hospitals (3 hubs and 4 spokes) in the Northern Italy Veneto region. The study was carried out in accordance with the Declaration of Helsinki (as revised in 2013) and the protocol was approved by the Comitato Etico per la Sperimentazione Clinica della Provincia di Padova (No. 5646/AO/23). All participating hospitals were informed and agreed to the study. Informed consent was obtained from each patient in the study.

Data collection

At least one leading pathologist and one referral pulmonologist were identified in each center and supported the collaboration. The three hubs for cancer care were designated on the base of performance criteria (volumes of specialized cancer surgery). The four spokes were connected to the hub hospital for selected services (e.g., administration of standard chemotherapies, diagnosis/staging by imaging techniques). All patients with NSCLC (early and advanced stages) diagnosed in each center were included in the study. Precise inclusion and exclusion criteria for pathological and clinical data were shared among PPUs (*Figure 1*). Clinical data included smoking history distinguishing current smoker (if they smoked until the month before diagnosis), former smoker (if they had stopped smoking at least one month before the diagnosis), and never-smoker, professional exposure, major symptoms, Eastern Cooperative Oncology Group (ECOG) performance status, diagnostic procedure, and pulmonary function tests that were available at the time of diagnosis. Pathological data comprised histo-type, type of specimen (surgical resection, biopsy, and cytology), biomarkers, and type of method used. The subgroup with complete clinical data was used to assess potential correlations between clinical and pathological findings (*Figure 1*).

The PPU of each center was responsible for filling an anonymous electronic case report form-Research Electronic Data Capture (REDCap®).

Data analysis

Data were reported as means and standard deviations for

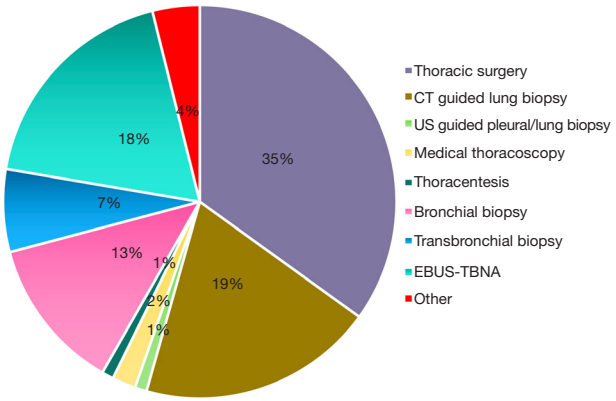

Figure 2 The diagnostic procedures used in the 7 centers. CT, computed tomography; US, ultrasound; EBUS-TBNA, endobronchial ultrasound-transbronchial needle aspiration.

continuous variables and as absolute values and percentages for categorical variables. Different tissue sources were compared using Pearson's chi-square test; P values were adjusted for multiplicity using the Benjamini-Hochberg correction.

Multivariable logistic regression analyses were employed to estimate the effects of various variables on the presence of mutations in different genes. The results were reported as odds ratios (ORs) with 95% confidence intervals (CIs). Statistical significance was set at P<0.05. Statistical analyses were performed using the R software version 4.3.1 (17).

Results

A total of 1,817 patients met the eligibility criteria for the primary goal of our study. The median age was 69 years (standard deviation ±10), the majority of patients were males (1,072, 59%) and in advanced stages 927 (51%) (further details are provided in Table S1).

Most patients had non-squamous histology (1,562, 86%), and a wide percentage were diagnosed with adenocarcinoma (1,308, 72%).

Diagnostic procedures and biomarker detection

The turnaround time from test orders to availability of results was similar among different PPUs, median time of 15.5 working days, according to local guidelines reported in our lung cancer care pathways (16).

Most of the specimens were cytological samples and small biopsies, including bronchial and transbronchial biopsies (43%). Surgical resections (35%) were mostly performed in hub hospitals (30% *vs.* 10%; P<0.001) (*Figure 2*).

When the different tissue sources were compared, a significantly different biomarker performance testing was found between cytological, biopsy, and surgical samples (*Table 1*).

As expected, surgical specimens showed a better performance for the detection of most biomarkers compared to cytology (P<0.001). The biopsy (both transbronchial/bronchial and CT-guided biopsy) performance was satisfactory compared to cytology which was significantly less reliable in the assessment of almost all the molecular targets. When the biopsy was compared with the surgical specimen, a similar performance was observed for PD-L1, *ALK* [both by immunohistochemistry (IHC) and molecular test], *BRAF*, and *KRAS* determination, whereas it was slightly worse for *EGFR*, *ROS1*, *MET*, and *RET* assessment (*Table 1*).

Overall biomarker testing rate

PD-L1 assessment was available in 91% of samples (n=1,653) with one third characterized by PD-L1 negative expression (<1%), another third showed mean PD-L1 expression (1≤ PD-L1 <49%) and the other third had high PD-L1 expression (≥50%). *EGFR* testing was performed on 1,411 patients (78%). The overall population had at least one biomarker test available. The results from all 5 biomarkers were available in 30% of the whole population and in 36% of the advanced NSCLC patients.

Results of all biomarkers and how they changed over time are reported in *Table 2* and *Figure 3*.

Testing with NGS was performed in 14% of the overall population and 16% of the advanced NSCLC cases. NGS testing was more often executed in hub than spoke hospitals (17% *vs.* 3%; P<0.001). Availability of NGS test results increased significantly over time (P<0.001), as shown in *Figure 4*.

Co-occurring biomarkers

Logistic regression models, adjusted for histo-type, were developed to investigate the association between PD-L1 expression and the mutational status of each oncogene. A significantly higher frequency of *ALK* rearrangement was found in samples with 1%≤ PD-L1 <49% and PD-L1 ≥50% compared to PD-L1 <1% [OR (95% CI): 2.56

Table 1 Biomarkers assessed per sample type

Biomarkers	Biopsy (N=1,060)	Cytology (N=215)	Surgical resection (N=516)	P value		
				Biopsy *vs.* cytology	Biopsy *vs.* surgical resection	Cytology *vs.* surgical resection
PD-L1				0.005**	0.07	<0.001***
Assessable	974 (99%)	177 (96%)	455 (100%)			
Not assessable	10 (1%)	8 (4%)	0 (0%)			
EGFR				<0.001***	0.02*	<0.001***
Assessable	773 (96%)	159 (83%)	390 (99%)			
Not assessable	31 (4%)	32 (17%)	4 (1%)			
ALK (IHC)				0.04*	0.07	<0.001***
Assessable	511 (98%)	78 (93%)	298 (100%)			
Not assessable	12 (2%)	6 (7%)	1 (0%)			
ALK				<0.001***	0.07	<0.001***
Assessable	401 (82%)	87 (58%)	136 (89%)			
Not assessable	87 (18%)	63 (42%)	17 (11%)			
ROS1 (IHC)				0.12	0.01*	<0.001***
Assessable	404 (97%)	75 (93%)	275 (100%)			
Not assessable	13 (3%)	6 (7%)	0 (0%)			
ROS1				<0.001***	0.02*	<0.001***
Assessable	491 (80%)	65 (43%)	176 (88%)			
Not assessable	124 (20%)	85 (57%)	23 (12%)			
BRAF				<0.001***	0.20	<0.001***
Assessable	530 (97%)	103 (74%)	221 (99%)			
Not assessable	14 (3%)	37 (26%)	2 (1%)			
RET				<0.001***	0.004**	<0.001***
Assessable	424 (74%)	64 (42%)	180 (86%)			
Not assessable	150 (26%)	87 (58%)	29 (14%)			
KRAS				<0.001***	0.07	<0.001***
Assessable	571 (96%)	111 (76%)	254 (99%)			
Not assessable	22 (4%)	36 (24%)	3 (1%)			
MET (IHC)				>0.99	>0.99	>0.99
Assessable	1 (33%)	3 (38%)	0 (NA)			
Not assessable	2 (67%)	5 (63%)	0 (NA)			
MET				<0.001***	0.005**	<0.001***
Assessable	484 (83%)	84 (55%)	208 (92%)			
Not assessable	101 (17%)	68 (45%)	18 (8%)			
NTRK (IHC)				<0.001***	>0.99	<0.001***
Assessable	94 (99%)	8 (62%)	66 (99%)			
Not assessable	1 (1%)	5 (38%)	1 (1%)			
NTRK1–3				0.90	0.20	0.10
Assessable	19 (16%)	7 (13%)	11 (27%)			
Not assessable	99 (84%)	46 (87%)	30 (73%)			

P value, Benjamini & Hochberg correction for multiple testing. *, P<0.05; **, P<0.01; ***, P<0.001. IHC, immunohistochemistry.

Table 2 Biomarkers assessment

Biomarker	%	Assessable, n [% of 1,817]
PD-L1		1,653 [91]
<1%	29	
1–49%	32	
≥50%	25	
Not assessable	5	
EGFR[1]		
Molecular test		1,411 [78]
NGS	24	
RT-PCR	76	
Molecular result		
Mutated	16	
Wild type	79	
Not assessable	5	
BRAF[2]		
Molecular test		925 [51]
NGS	35	
RT-PCR	64	
Molecular result		
Mutated	5	
Wild type	89	
Not assessable	6	
KRAS[3]		
Molecular test		1,020 [56]
NGS	33	
RT-PCR	67	
Molecular result		
Mutated	34	
Wild type	60	
Not assessable	6	
RET[4]		
Molecular test		955 [53]
NGS	15	
RT-PCR	85	
Molecular result		
Mutated	2	
Wild type	70	
Not assessable	28	
MET[5]		
Molecular test		986 [54]
NGS	27	
RT-PCR	73	
Molecular result		
Mutated	4	
Wild type	77	
Not assessable	19	
ALK		
Immunohistochemistry score		917 [50]
0	92	
1	2	
2	2	
3	2	
Not assessable	2	
Molecular test		750 [41]
FISH	7	
NGS	16	
RT-PCR	77	
Molecular result		
Mutated	4	
Wild type	75	
Not assessable	21	
ROS1		
Immunohistochemistry score		784 [43]
0	79	
1	6	
2	9	
3	3	
Not assessable	3	
Molecular test		979 [54]
FISH	22	
NGS	14	
RT-PCR	64	

Table 2 (*continued*)

Table 2 (*continued*)

Table 2 (*continued*)

Table 2 (*continued*)

Biomarker	%	Assessable, n [% of 1,817]
Molecular result		
Mutated	1	
Wild type	75	
Not assessable	24	
NTRK1–3		
Immunohistochemistry		178 [10]
Positive	2	
Negative	94	
Not assessable	4	
Molecular test		212 [12]
FISH	10	
NGS	49	
RT-PCR	41	
Molecular result		
Mutated	0	
Wild type	17	
Not assessable	83	

[1], the most common *EGFR* aberrancy were exon 19 deletion (51%) and exon 21 *L858R* mutation (37%). [2], the *BRAF* V600E mutation was most commonly detected. [3], G12C was the most frequently detected *KRAS* alteration. [4], exon 12 fusions were the main *RET* aberrancies detected. [5], MET exon 14 skipping skipping alteration was more frequently detected. FISH, fluorescent in situ hybridization; NGS, next-generation sequencing; RT-PCR, reverse transcriptase-polymerase chain reaction.

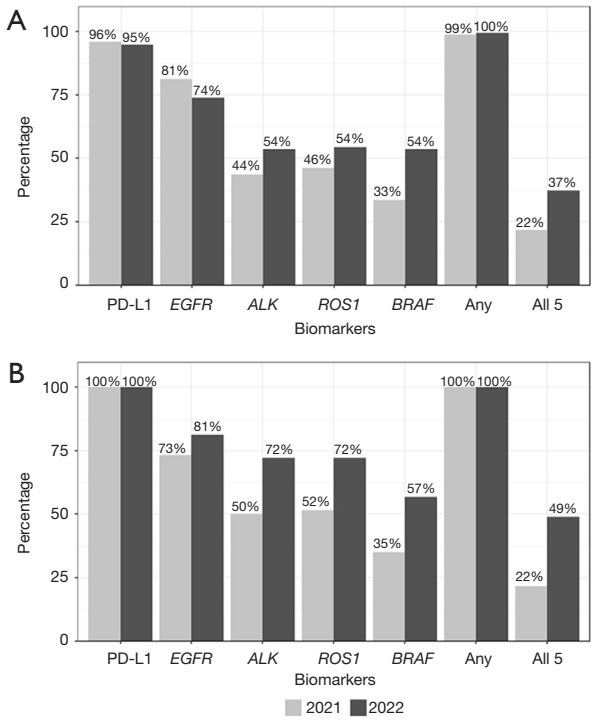

Figure 3 Biomarker testing patterns over time. (A) Overall. (B) Advanced stage.

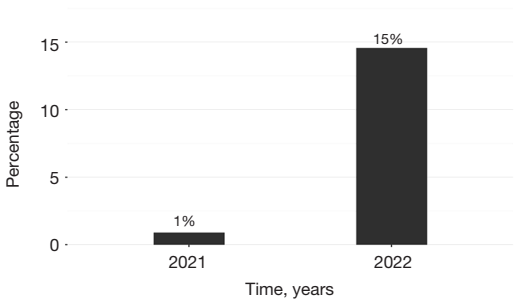

Figure 4 Next generation sequencing testing across the 2021–2022 observation time. P value <0.001 for trends over time. P value was calculated from Cochrane Armitage test for trend analysis.

(1–7.57); P=0.04 and OR (95% CI): 3.59 (1.42–10.56); P<0.001, respectively]. Of interest, PD-L1 >50% was associated to increased risk of *MET* [OR (95% CI): 2.55 (1.07–6.57); P=0.03], *KRAS* [OR (95% CI): 1.73 (1.27–2.36); P<0.001] and *BRAF* [OR (95% CI): 2.63 (1.18–6.35); P=0.02] mutations. High PD-L1 expression (>50%) was rarely detected in *EGFR*-mutated cases [OR (95% CI): 0.33 (0.22–0.49); P<0.001].

Among the 1,817 samples examined, at least one molecular aberrancy was detected in 742 (41%). Co-occurring molecular targets were detected in 42 cases (2%) (*Figure 5*). NGS testing was applied in 34% of the samples harboring 2 or more molecular targets and in 14% of those with one or none (P=0.001). Among these, the most common were the concomitant alterations of *ALK* and *KRAS* (n=3), *MET* and *KRAS* (n=4), *BRAF* and *KRAS* (n=6). Further details are listed in Table S2.

Association between pathological/molecular data and clinical findings

Former smokers [OR (95% CI): 0.6 (0.41–0.91); P=0.02]

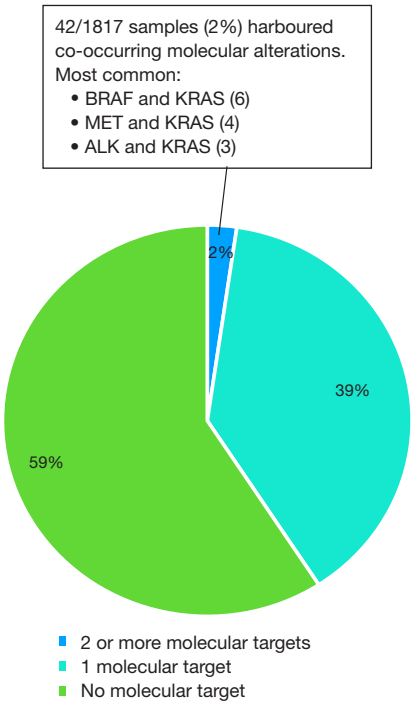

Figure 5 The occurrence of molecular abnormalities in the study population.

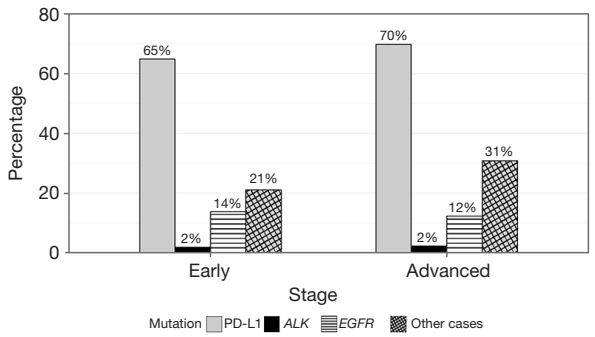

Figure 6 Mutation prevalence across stages. Prevalence of PD-L1 1–49% and PD-L1 >50% cases, *EGFR*-mutated cases, *KRAS*-mutated cases and other cases in early and advanced stages. No difference was found in PD-L1, *ALK* and other biomarkers expression across the disease stages. PD-L1, programmed death ligand 1.

and never-smokers [OR (95% CI): 0.6 (0.37–0.99); P=0.048] had less probability to show PD-L1 expression >50% than current smokers.

Patients with hemoptysis at the time of diagnosis shared a higher probability of PD-L1 staining >50% than asymptomatic ones [OR (95% CI): 1.98 (1.06–3.64); P=0.02].

ALK rearrangement was more common among non-smokers than current smokers [OR (95% CI): 7.29 (1.78–49.3); P=0.01].

Patients complaining pain at the time of diagnosis had a higher probability of sharing *ALK* alterations than asymptomatic patients [OR (95% CI): 2.68 (1.03–6.19); P=0.02].

MET abnormalities were more common in patients with respiratory failure than normoxic ones [OR (95% CI): 6.67 (0.92–32.1); P=0.02].

Males had a lower probability of harboring *EGFR* abnormalities than females [OR (95% CI): 0.28 (0.19–0.4); P<0.001]. The probability of *EGFR* mutations was higher in asymptomatic never-smokers compared to current smokers [OR (95% CI): 3.75 (1.96–7.51); P<0.001] and symptomatic patients [OR (95% CI): 1.58 (1.06–2.532); P=0.02].

KRAS aberrancies were more common in patients with professional exposure to pneumotoxic agents [OR (95% CI): 2.07 (1.12–3.85); P=0.02] and in both current [OR (95% CI): 4.41 (2.24–9.38); P<0.001] and former smokers [OR (95% CI): 4.12 (1.97–9.19); P<0.001] compared to never-smokers.

The risk of neurologic symptoms at diagnosis was higher in *BRAF*-mutated patients [OR (95% CI): 3.7 (1.2–9.53); P=0.01].

A trend was detected when the association between squamous cell carcinoma and high PD-L1 expression was analyzed [OR (95% CI): 1.33 (0.99–1.78); P=0.06].

The probabilities of detecting PD-L1 expression >50%, any genomic aberrancies, and at least one targetable alteration were similar when the early stages were compared to advanced stages (*Figure 6*).

Two or more concomitant molecular targets were more frequent among women than men (58% *vs.* 42%; P<0.001), never-smokers compared to current smokers (50% *vs.* 6.3%; P<0.001), and patients without dyspnea than patients complaining of shortness of breath (15% *vs.* 6%; P=0.01).

The co-occurrence of three different molecular abnormalities was detected in three early-stage adenocarcinomas: (I) *MET* [NM_001127500.2(MET):c.3029C>T (p.Thr101Ile)], *BRAF* [NM_004333.4(BRAF):c.1391G>T (p.Gly464Val)], and *PIK3CA* [NM_006218.3(PIK3CA):c.1624G>A (p.Glu542VLys)] in a 73-year-old former smoker with stage Ib adenocarcinoma; (II) *MET* [MET exon-14-skipping], *EGFR* [NM_005228.5(EGFR):c.2573T>G (p.Leu858Arg)], and *KRAS* [NM_004985.5(KRAS):c.35G>A (p.Gly12Asp)]

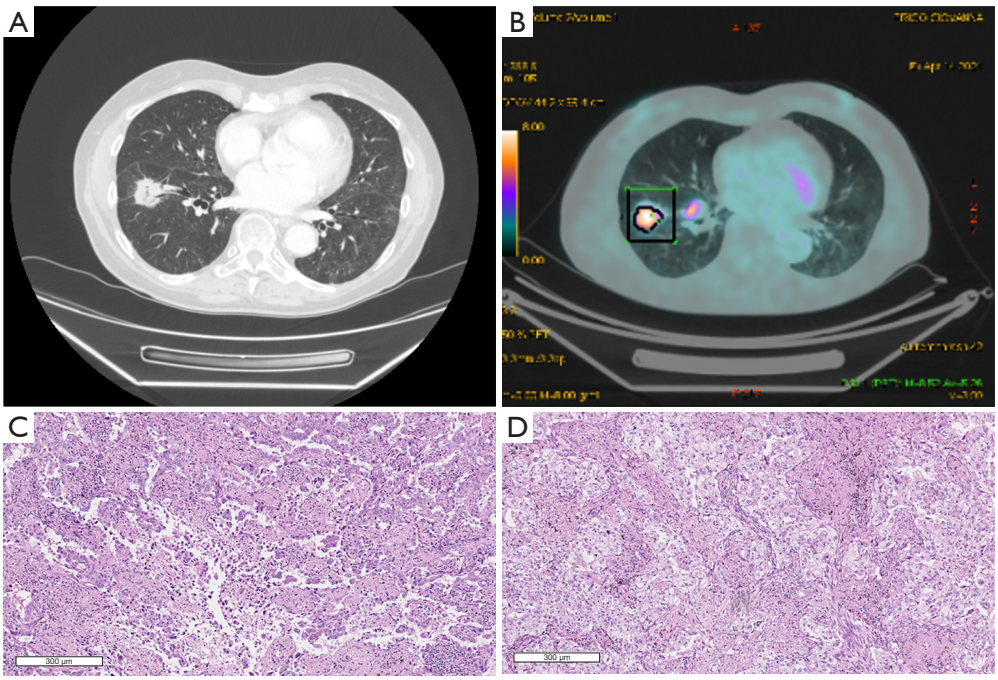

Figure 7 Explanatory case of patient with 3 co-occurring gene alterations (*MET*, *EGFR* and *KRAS* mutations). (A) The tumor at CT scan; (B) strong uptake of the tumor and the homolateral hilar lymph node at 18-FDG PET/CT; (C,D) microscopical findings show heterogeneous morphological features of adenocarcinoma with acinar (C) and solid (D) growth patterns. Scale bar: 300 µm. Staining: haematoxylin and eosin.

in a 77-year-old woman who had never smoked and had no significant medical history with surgically resected stage IIIa adenocarcinoma (*Figure* 7); (III) *KRAS* [NM_004985.5(KRAS):c.34G>A (p.Gly12Ser)], *MET* [NM_020975.6(RET):c.3116C>T (p.Pro1039Leu)], and *BRAF* [NM_004333.6(BRAF):c.1390G>A (p.Gly464Arg)] in a 63-year-old man with surgically resected stage Ib adenocarcinoma and history of thyroid and kidney cancers.

Discussion

In this real-world study of an unselected multicenter cohort of patients with NSCLC, we documented the diagnostic journey mainly focusing on molecular assessment by PPUs of hub and spoke hospitals, according to our local lung cancer care pathway (16).

PD-L1 expression was assessed in over 90% of samples while the oncogenes *EGFR*, *ALK*, *BRAF* and *ROS1* were evaluated in about 50% (78%, 50%, 43% and 51%, respectively) of the overall study population. This rate was similar to the metastatic NSCLC of the US oncology network (18), with the simultaneous assessment of all 5 essential biomarkers achieved in a similar percentage of our advanced cases, namely 22–49% *vs.* 33–46% in the study by Robert *et al.*

These low percentages probably reflect the screening in fewer representative samples as cytologies and small biopsies, even with the advent of tissue sparing procedures like NGS which, anyway, was increasingly applied in our centers over time. Indeed, even if several pathology laboratories are now equipped for cytology smear analysis and cytoblock evaluation of biomarkers either *in situ* by immunohistochemistry or molecular analyses their performance has often been reported to be limited (19,20) as it also turned out in our study. *NTRK1–3* fusions were investigated only in 212 (12%) samples as the target treatment was recently approved in NSCLC assessment (5).

Across the study period, a stable percentage of samples was tested for PD-L1 while oncogene assessment had a steep increase between the first months of 2021 and the following time points.

The accessibility of testing facilities and healthcare services during periods of heightened pandemic-related restrictions could have posed barriers to patient

engagement with biomarker testing. Factors such as limited transportation options, reduced clinic hours, and prioritization of resources for COVID-19 testing and treatment may have inadvertently impeded access to NSCLC biomarker testing for some individuals (21). However, it is essential to acknowledge that the decline in testing rates is likely influenced by a combination of factors, reflecting the multifaceted nature of healthcare delivery during the pandemic.

In our study, 41% of patients harbored a driving molecular aberrancy. Among those, a small but relevant percentage (2%) shared two or more co-occurring genomic alterations more often detected by NGS whose use was progressively implemented in the last months of our study (22). The occurrence of multiple genomic aberrancies in NSCLC is potentially more impactful than distinct mutations in oncogenic drivers and represents a challenge for medical oncologists who are asked to choose the most appropriate target of treatment and further personalize patient management (23). In our study, EGFR mutant adenocarcinomas showed a significantly lower frequency of PD-L1 >50%. The link between the *EGFR* signaling pathway and PD-L1 expression in NSCLC is currently under investigation (24). Further studies will explain the inefficacy of anti-PD-1/PD-L1 immune check-point inhibitors compared to chemotherapy in advanced *EGFR*-mutant NSCLC (25) and of the combination EGFR-tyrosine kinase inhibitor (TKI) plus anti-PD-1 inhibitor compared to EGFR-TKI monotherapy (26).

It is noteworthy that the three patients who showed multiple concomitant genomic alterations had all been diagnosed with early-stage adenocarcinomas. Evidence of the frequency (27) and the importance (7,28) of *EGFR* mutation in the early stage is accumulating. Osimertinib, an oral EGFR-TKI, effectively improves disease-free survival, reduces the risk of local and distant recurrence after complete tumor resection (ADAURA 1 and 2 trials), and is now being tested as a neoadjuvant approach for resectable stage II–IIIb N2 *EGFR* mutated lung cancers (29). Nonetheless, since the advent of NGS, it has become increasingly evident that treatment response is far from being homogeneous even in target therapy. Such variability, along with *de novo* resistance, could be linked to the molecular intra-driver heterogeneity (30). In this context, our observations support the importance of accurate molecular profiling even in the early stage of NSCLC as well. Recently, a few real-world results from other centers have already underlined the importance of comprehensive tumor characterization, including early-stage adenocarcinoma, to better define the clinical/molecular phenotypes, to guide the following therapy choices (31-33).

Clinical-pathological and molecular correlations revealed intriguing findings, some confirming data already reported in the literature and others expanding previous knowledge, that could be helpful for more appropriate management of patients. Never-smoking women with minor symptoms had the highest probability of harboring coexistent aberrancies which included not only *EGFR*, as expected based on a large volume of literature, but also *KRAS* and *MET* mutations, usually found in smokers. Of interest, smoking-related molecular aberrancies were found in never smokers in a recent genome-wide study suggesting that clinically based classification of NSCLC could be biased (34). Along this line, our data indicate the importance of deep molecular profiling even in early-stage settings, regardless of clinical characteristics such as smoking history. Further research and analysis are needed to fully understand how these associations actually translate into actionable clinical insights and influence patient care.

In our cohort, squamous cell carcinoma patients who were active smokers and had hemoptysis showed high expression of PD-L1. On the link between PD-L1 and smoking history, contradictory results have been reported in previous studies (35,36), perhaps because former/current smokers were not distinguished and because of the co-occurrence of genetic and epigenetic mechanisms. Cigarette smoke has been shown to induce PD-L1 expression on lung epithelial cells *in vitro* and *in vivo* by the selective link between the carcinogen benzo(a)pyrene and the aryl hydrocarbon receptor (37).

On the other hand, 7% of our nonsmoker adenocarcinoma cases showed the typical *ALK* translocation, a carcinogenic abnormality shared by several different cancers which, like *EGFR* mutations (38), is less common in patients exposed to cigarette smoke (39).

Supporting the previously suggested link between *KRAS* mutation and occupational asbestos exposure in lung adenocarcinoma (40), we found a higher frequency of *KRAS* aberrancies among NSCLC patients with professional exposure to pneumotoxic agents (mainly asbestos).

The presence of neurologic symptoms at diagnosis was common in our *BRAF*-mutated cases, whose association between brain metastasis and specific mutations has been described (41). In this large case series of *BRAF*-mutant

NSCLC, almost 1/10 patients with V600E mutation and extrathoracic localizations had brain metastasis. Even higher percentages were detected in patients with different functional classes of *BRAF* mutations, poorly represented in our cohort.

A limitation of our work is that some patient variables were missing due to the observational and retrospective design of the study. However, the high number of cases and data collected by different centers has provided a reliable portrait of the biomarker landscape of NSCLC in the Veneto region. This indicates the need for an effective collaboration not only between different centers (hub and spoke) but above all the different PPUs with consolidated activities. Indeed, in the effort to provide quality data for the study, the participating researchers/personnel frequently met, shared experiences and difficulties. Moreover, the team was used to find solutions for practical limitations, strengthening the link between the specialists of the NSCLC diagnostic pathway. Along with depicting the pathologic and clinical phenotype of the patients diagnosed with NSCLC, this study represents an example of self-criticism and growth for integrated care teams.

Conclusions

This real-world study of patients diagnosed with NSCLC, the majority of whom were tested for biomarkers, was conducted in a regional network of pathologists and pulmonologists. Due to the advent of new molecular testing techniques, the evaluation of complementary genomic aberrancies has progressively increased. Complex advanced NSCLC cases requiring complete molecular profiling could benefit from being referred to hub centers where lung surgery and NGS testing techniques are suitable and standardized. Clinical-molecular phenotypes, if confirmed in future larger studies, could give insights not only into patient management but also for investigating new pathogenetic pathways.

Acknowledgments

We thank the whole Thoracic Oncology Multidisciplinary Team and, overall, all our patients.

Funding: This work was supported by the Profiling Patients with Non-Small Cell Lung Cancer: Diagnostic and Prognostic Stratification According to Clinical, Functional and Immunological Pattern (BIRD221905/22) and the unrestricted educational grant ROCHE "GRUPPO di MIGLIORAMENTO: MANAGEMENT DEL PAZIENTE CON TUMORE POLMONARE".

Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-107/rc

Data Sharing Statement: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-107/dss

Peer Review File: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-107/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-107/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 carried out in accordance with the Declaration of Helsinki (as revised in 2013) and the protocol was approved by the Comitato Etico per la Sperimentazione Clinica della Provincia di Padova (No. 5646/AO/23). All participating hospitals were informed and agreed to the study. Informed consent was obtained from each patient in the study.

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Cite this article as: Tinè M, Pezzuto F, Orlandi M, Caliò A, Marletta S, Bondavalli T, Giongo D, Reghellin D, Posenato I, Santacatterina M, Menin A, Welte PK, Baciorri F, Sacchi D, Catino C, Nicolè L, Foltran G, D'Urso A, Orzes N, Andreotti G, Bartolotta P, Gregori D, Pasello G, Rea F, Calabrese F. Real-world biomarker testing patterns: clinical-pathological portrait of early and late non-small cell lung cancer in hub and spoke North Italian centers. Transl Lung Cancer Res 2024;13(7):1505-1517. doi: 10.21037/tlcr-24-107