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Review Article

# Pathologic Assessment and Staging of Multiple Non–Small Cell Lung Carcinomas: A Paradigm Shift with the Emerging Role of Molecular Methods

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ABSTRACT

Non–small cell lung carcinomas (NSCLCs) commonly present as 2 or more separate tumors. Biologically, this encompasses 2 distinct processes: separate primary lung carcinomas (SPLCs), representing independently arising tumors, and intrapulmonary metastases (IPMs), representing intrapulmonary spread of a single tumor. The advent of computed tomography imaging has substantially increased the detection of multifocal NSCLCs. The strategies and approaches for distinguishing between SPLCs and IPMs have evolved significantly over the years. Recently, genomic sequencing of somatic mutations has been widely adopted to identify targetable alterations in NSCLC. These molecular techniques have enabled pathologists to reliably discern clonal relationships among multiple NSCLCs in clinical practice. However, a standardized approach to evaluating and staging multiple NSCLCs using molecular methods is still lacking. Here, we reviewed the historical context and provided an update on the growing applications of genomic testing as a clinically relevant benchmark for determining clonal relationships in multiple NSCLCs, a practice we have designated "comparative molecular profiling." We examined the strengths and limitations of the morphology-based distinction of SPLCs vs IPMs and highlighted pivotal clinical and pathologic insights that have emerged from studying multiple NSCLCs using genomic approaches as a gold standard. Lastly, we suggest a practical approach for evaluating multiple NSCLCs in the clinical setting, considering the varying availability of molecular techniques.



## Introduction

The widespread use of low-dose computed tomography (CT) scans for lung cancer screening in smokers and cancer surveillance has led to a significant increase in the detection of early-stage lung cancers, including increased detection of patients with multiple lung cancers. Studies suggest that 20% to 25% of smokers undergoing CT-based screening have 2 or more lung cancers detected.[1-4] At Memorial Sloan Kettering Cancer Center (MSKCC), more than 20% of patients who have undergone surgery in the recent decade had 2 or more synchronous or metachronous non–small cell lung carcinomas (NSCLCs) (NR and JC, personal observations). Determining whether multiple NSCLCs represent separate primary lung carcinomas (SPLCs) or intrapulmonary metastases (IPMs) of a single tumor has been a long-standing clinicopathologic dilemma. Establishing robust diagnostic tools to differentiate between these distinct biological processes is a critical issue, and is increasingly relevant given the high prevalence of patients with multiple NSCLCs in current practice.

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The methods and criteria for distinguishing SPLCs and IPMs have evolved over time. In recent years, genomic testing of somatic mutations has become a widely used method for identifying targetable alterations in NSCLC. In parallel, genomic methods have also emerged as powerful and clinically accessible tools for assessing clonal relationships among multiple NSCLCs. However, genomic methods have not been incorporated in a standardized way into the assessment and staging of multiple NSCLC cases. In this concise review, we discussed the historical background and recent developments in genomic testing emerging as a robust and clinically applicable gold standard for establishing clonal relationships among multiple NSCLCs. We also highlighted several key clinicopathologic lessons that have emerged from the analysis of multifocal NSCLCs using genomic testing and illustrated strengths and weaknesses of pathologic assessment of multiple tumors. Finally, we proposed a practical approach to the assessment of multiple NSCLCs in clinical practice, taking into account different levels of access to molecular methodologies.

## Historical Approaches to the Assessment of Multiple Non–Small Cell Lung Carcinomas

Martini and Melamed[5] were the first to propose a set of clinical and pathologic criteria for the distinction of SPLCs and IPMs. They defined SPLCs as tumors consisting of different histotypes (such as adenocarcinoma vs squamous cell carcinoma vs small cell carcinoma) or as tumors with features that make metastasis unlikely, such as tumors located in different lobes without lymphovascular invasion and nodal or extrapulmonary metastases. Subsequently, the American College of Chest Physicians (ACCP) added a time interval of more than 2 years as an additional criterion arguing against IPMs and favoring SPLCs.[6]

Although the Martini and Melamed criteria were able to identify patients with more aggressive tumors, in whom second lung tumors are statistically more likely to be metastasis than a new primary, the lack of evidence for distant or nodal metastases does not exclude the possibility of intrapulmonary spread. Another major limitation of these criteria is that in recent decades, the vast majority of multiple lung cancers are adenocarcinomas, reducing the relevance of different histotypes as the major distinguishing feature in SPLCs.

Subsequently, Girard et al[7] proposed comprehensive histologic assessment as a method to enable a more granular and accurate comparison of multiple NSCLCs. This approach involved not only the comparison of overall histologic subtype but also detailed histologic features of tumors, including architectural patterns in adenocarcinoma, cytologic features, and stromal features.

In subsequent studies, using molecular methods as a gold standard, the Martini and Melamed[5] criteria were discrepant with molecular results in 28% to 50% of cases.[7-10] Although comprehensive histologic assessment has been shown to be effective in most cases, recent molecular studies have shown that it can be inaccurate in approximately 20% (range 11%-39%) of cases.[9-17]

## Staging of Multiple Non–Small Cell Lung Carcinomas

The designation of tumors as SPLCs vs IPMs has major implications for tumor staging. In the current eighth edition of the American Joint Committee on Cancer (AJCC) Staging Manual, IPMs are staged as T3 if tumors are located in the same lobe, T4 if located in a different ipsilateral lobe, and M1a if located in the contralateral lung, whereas SPLCs are staged individually based on individual tumor parameters.[18] The criteria for separating SPLCs and IPMs in AJCC staging have evolved over the editions, with the AJCC fifth edition and earlier editions relying solely on clinicopathologic parameters (Martini and Melamed criteria). The sixth edition mentioned mutation testing but did not provide specific criteria. The seventh edition suggested the use of “breakpoint” analysis, which requires mate-pair sequencing on fresh frozen tissue, which is not a widely applicable assay for formalin-fixed paraffin-embedded-based clinical practice.

Since the publication of the seventh AJCC manual in 2010, significant knowledge has accumulated on the utility of driver gene- and next-generation sequencing (NGS)-based molecular testing for determining the clonal relationships of multifocal NSCLCs. As a result, significant updates to the AJCC manual that incorporate molecular parameters can be anticipated.

## Evolution of Molecular Approaches for the Assessment of Multiple Non–Small Cell Lung Carcinomas: Broad-Panel Next Generation Sequencing as an Emerging Gold Standard

A variety of molecular techniques have been applied for evaluating multifocal NSCLCs over the years, including loss of heterozygosity analysis, array comparative genomic hybridization, *TP53* gene mutation status, and NGS-based genomic breakpoint analysis.[7,19-26] Most of these molecular assays were conducted in research laboratory settings or required fresh tissue rather than formalin-fixed paraffin-embedded tissue. The advent of genomic mutation testing as a routine clinical method for predictive assessment of NSCLC has marked a major shift in the approach to clonality assessment. Although the primary goal of molecular testing is to identify targetable genomic alterations, it opened access to sequencing technologies in routine clinical care, thus providing an unprecedented opportunity to assess the clonal relationships among NSCLCs at scale.

In the pre-NGS era, routine molecular testing analysis for NSCLC encompassed several major mitogenic driver alterations, including *EGFR, KRAS, ALK*, and *ROS1* using various single-gene or small oligo-gene (eg, 4-gene) panel methodologies available in clinical practice, including Sanger sequencing, PCR, and fluorescence in situ hybridization, among others.[8,9,11,27,28] It is currently well established that these major driver alterations in NSCLC represent early clonal truncal events: if present, the driver alterations are present in all tumor cells and can therefore be reliably used as clonality-defining events.[29] Using this principle, the presence of distinct driver alterations in lung adenocarcinomas (such as *EGFR* in one tumor and *KRAS* in another) can be used to classify a tumor pair as SPLCs (clonally unrelated). However, driver-only analysis may not always be informative or fully conclusive in a significant number of cases (see the section on NGS vs driver-only testing below). Of note, early studies utilizing Sanger sequencing and other lower-sensitivity methods have occasionally identified discrepant driver-gene status among different samples from presumably the same NSCLC; these findings are likely attributable to the limited analytical sensitivity of these assays in samples with borderline tumor content—a common issue in lung cancer given their frequent association with significant tumor-associated inflammatory infiltrate.

Subsequently, with the increasing number of targetable genomic alterations identified in NSCLC, multigene NGS panels have become an increasingly used method in clinical practice.

These include medium-sized panel platforms that interrogate hotspot mutations only, such as amplicon sequencing-based panels (eg, 50-gene Ampliseq panel and 50-gene Oncomine panel) and multiplexed PCR-based assays (eg, 11-gene AmoyDx panel).[10,12,14,16,30-32] More recently, broad-panel NGS approaches have entered the clinical arena. These panels interrogate hundreds of cancer-related genes simultaneously and test not only hotspot mutations but also various other types of genomic alterations, including protein-altering nonhotspot mutations, silent (synonymous) mutations, intronic mutations, as well as copy number alterations and structural variations. This results in a highly granular genomic profile that can serve as a virtually unique "fingerprint" of an individual tumor. In our practice, we have adopted the term "comparative molecular profiling" as an approach for determining whether 2 tumors are related (IPMs) or unrelated (SPLCs).

To date, several studies, including one from MSKCC, have explored the utility of broad-panel NGS for subtyping multiple NSCLCs in Western and East Asian populations.[13,17,33-35]

In the following paragraphs, we discuss emerging insights regarding clinical, pathologic, and molecular features of multiple NSCLCs based on recent broad-panel NGS studies, and the implications for more targeted diagnostic approaches.

## Major Lessons for Molecular Assessment of Multiple Non–Small Cell Lung Carcinomas by Broad-panel Next Generation Sequencing

(1) *In most cases, the assessment of multiple NSCLCs is straightforward by broad-panel NGS without requiring special bioinformatics methods.*

As mentioned above, broad-panel NGS provides a highly granular view of a tumor's mutational profile, allowing a robust comparison of the clonal relationship between NSCLCs. IPMs typically harbor multiple shared somatic alterations, whereas SPLCs have unique mutational profiles, enabling a confident classification as such.

Despite the potential for clonal evolution and the acquisition of additional mutations during metastasis, the truncal nature of most mutations in NSCLC allows a core number of shared mutations to serve as a robust marker of tumor clonality.[13,36] Similarly, complete nonoverlap of genomic profile can serve as strong evidence of distinct clonal relationships, barring the issue of coincidentally shared common hotspot mutations, which is discussed later.

Using these principles, in our study from MSKCC that included 76 tumor pairs analyzed by broad-panel NGS, virtually all cases could be readily classified by manual review as unambiguous SPLCs or IPMs.[13] The high-technical sensitivity of NGS allowed us to analyze tumors with low tumor purity and still obtain a sufficient number of mutations for clonality comparison. Only one out of 76 tumor pairs showed equivocal results due to the low number of mutations present in each tumor.

The subsequent study that utilized broad-panel NGS for multiple NSCLCs comparisons was by Yang et al[17] and included analysis of 42 NSCLC pairs in East Asian patients. This cohort was enriched in never-smokers with tumors harboring *EGFR* mutations and overall lower tumor mutation burden— characteristics that reflect the distinct lung cancer biology in East Asian patients with NSCLC. Despite this, clonality assessment was still feasible in the majority of cases and demonstrated the effectiveness of broad-panel NGS in multiple NSCLC assessments.

Although these initial studies focused on the clonality assessment predominantly in patients with multiple adenocarcinomas, a recent study using whole exome sequencing of 20 pairs of lung squamous cell carcinomas also showed that nearly all cases could be readily classified as unambiguous SPLCs or IPMs.[35]

In our experience, comparative molecular profiling using clinical-grade broad-panel NGS can generally be made by manual review of alterations reported in standard molecular reports, without requiring special bioinformatic approaches. However, this statement is predicated on the use of paired tumor-normal sequencing, whereas tumor-only NGS may require significant technical expertise in interpretation, as will be discussed further in major lesson 3 in this section.

(2) *Separate primary tumors can sometimes coincidentally harbor the same common driver mutation (such as KRAS* G12C*).*

This issue highlights the major limitation of using driver-only panels to determine NSCLC relatedness. SPLCs in Western countries most commonly arise in current or former smokers, where mutations, such as *KRAS* G12C occur at a relatively high frequency (~24%). As a result, the odds of 2 SPLCs harboring this mutation by chance are as high as 1 in 17. Similarly, in nonsmokers and in East Asian countries where *EGFR* mutations account for almost 50% of driver alterations in NSCLC, the chances of 2 SPLCs harboring identical, common *EGFR* variants such as L858R or E746_A750del can be as high as 1 in 12. Therefore, a shared common driver alone should not be taken as definitive evidence of a clonal relationship. Broad-panel NGS and WES-based studies clearly demonstrate that SPLCs can have shared common drivers yet have entirely nonoverlapping genomic profiles otherwise.[13,17,29] The interpretation of such cases by different assays are further discussed below.

For most cases reported to date, interpretation of cases with a single shared driver as SPLCs has been straightforward by broad-panel paired tumor/normal NGS given multiple unique mutations detected in each tumor and the absence of any additional shared mutation (summarized in Supplementary Table S1). However, as discussed next, there can be limitations with interpretation cases with shared drivers in the background of only a few unique mutations; continued accumulation of data will be needed to refine the approach to this scenario.

(3) *Broad-panel NGS has some limitations.*

First, NGS may not be informative if the tumor is extensively necrotic or if there is significant tumor-associated inflammation, leading to a decrease in tumor content below the assay's analytical sensitivity, which is typically approximately 5%-10% in standard NGS assays. In such cases, there may be either complete absence or paucity of detected alterations. A low total number of mutations, limiting comparative molecular profiling, may also be encountered in never-smokers with major tyrosine kinase drivers (eg, *EGFR*, *ALK*).

In such cases, it may be highly informative for molecular pathologists to examine alterations that are typically excluded by bioinformatic pipelines because they do not lead to protein alterations, but which are highly informative for clonality comparisons. This includes silent mutations or intronic mutations. Comparison of copy number profiles may also be informative.

In particular, this approach can be highly informative in scenarios where tumor pairs share a common driver, such as shared *EGFR* L858R, but lack other shared or unique mutations.[13,17] In these cases, identifying additional shared silent/intronic mutations would provide support for IPM. However, in cases with borderline tumor content, such scenarios may remain equivocal even after manual review (see Supplementary Table S1).

Another potential limitation of broad NGS panels is the need to account for non–tumor-derived variants if matched normal tissue is not sequenced alongside tumor tissue. Currently, tumor-only sequencing is the dominant practice in clinical sequencing. This approach, however, carries the risk of mistaking rare germline variants or clonal hematopoiesis mutations as shared tumor-specific mutations in comparative molecular profiling.[37,38] Although reference databases, such as dbSNP and gnomAD, may help identify relatively common germline variants, they may not document rarer variants.[39,40] In cases where uncertainties remain, retrospectively sequencing matched normal tissue (preferably blood) can help clarify whether a variant is somatic, germline, or clonal hematopoiesis-related in nature.

In summary, comparative molecular profiling can generally be effectively accomplished by surgical pathologists for cases with matched tumor/normal NGS and adequate tumor content. However, to ensure the most accurate and robust integration of pathologic and genomic results, collaboration between surgical and molecular pathologists is essential.

## Major Lessons for Panel Selection: Broad-panel NGS Vs Smaller Molecular Panels

Broad-panel NGS is required for some but not all cases**.** Although broad-panel NGS is highly effective for clonality assessment, its cost and availability may limit its universal applicability. An essential question then arises: when are smaller molecular panels, such as those interrogating only key mitogenic driver genes (such as *EGFR, KRAS, ALK, ROS1*) sufficient for clonality comparison?

As discussed above, in lung adenocarcinoma, these major drivers represent truncal events present in all tumor cells in primary and metastatic tumors, making them reliable markers for clonality. As a result, lung adenocarcinomas with distinct drivers can be classified as definite SPLCs without the need for further testing. Other than this specific scenario of distinct drivers being detected in each tumor, interpretation of all other combinations of driver-only analysis has limitations or is entirely inconclusive, as outlined below:

(1) *Driver absent in both tumors.* In the MSKCC study, based on broad-panel NGS assay, this scenario was encountered in 12% of tested patients with multiple NSCLCs; and in other studies, this scenario ranges from 14% to 24%.[10,11,13,17] For multiple lung squamous cell carcinomas, this scenario is virtually universal because such tumors generally lack major mitogenic drivers, such as *EGFR* or *KRAS*.[35] Although comparative molecular profiling by broad-panel NGS is highly effective in the absence of a driver, such cases remain entirely inconclusive by driver-only assays and may remain inconclusive by small-panel NGS panels.

(2) *Driver absent in one tumor vs present in the other.* Although this profile suggests that the tumors are unrelated, in driver-only testing, there is generally no built-in "internal control" to exclude a false-negative result due to low/borderline tumor content. In contrast, the absence of any mutations (including silent/intronic by broad-panel NGS) can serve as a "red flag" for inadequate tumor content. As a result, the absence of mutations in driver-only testing can sometimes represent an unsuspected false-negative result. To accept this result as supporting SPLCs, adequate tumor content in a driver-negative case should be histologically confirmed. Given that tumor-associated inflammation is common in NSCLC, in tumors with borderline tumor content it may be difficult to reliably exclude a false-negative result. In the MSKCC study, the combination of driver present vs driver absent accounted for 28% of tumor pairs.[13]

(3) *Shared single driver.* As discussed above, the presence of a shared driver suggests a likely clonal relationship; however, identical driver alterations may occur in 2 unrelated tumors by chance. Single-gene assays cannot resolve this. In contrast, broad-panel NGS generally provides either fully conclusive evidence of clonal relationship by identifying multiple shared mutations, which virtually eliminates the possibility of coincidental co-occurrence, or identifies multiple unique mutations, indicative of SPLCs. The probability of a single shared driver occurring by chance is dependent on the prevalence of the mutation in a given population. For example, as discussed above, shared common drivers, such as *KRAS* G12C in current/former smokers or *EGFR* L858R in never-smokers are unreliable indicators of tumor relatedness in isolation.[13,17] However, a shared rare alteration (prevalence <5% of the population) can be considered stronger evidence for IPM. Therefore, for driver-only testing, the interpretation of shared drivers requires the knowledge of mutation prevalence and is probabilistic rather than fully definitive. In the MSKCC study, coincidentally shared common drivers were identified in 9% of SPLCs.[13]

The above illustrates how broad-panel NGS can be used to predict the performance of single-gene or smaller panels. Using the cohort from our study as a model, we can estimate that driver-only testing would provide a definite determination of multiple NSCLC relationships (distinct drivers in each tumor) in 30% of cases, which is in line with what is reported in driver-only studies in various cohorts.[10,11] For an additional approximately 50%, a "likely" interpretation of tumor relationship could be rendered (driver present vs absent, or shared uncommon drivers); the degree of confidence would vary depending on specific pathologic and molecular variables of the individual cases. Lastly, approximately 20% would remain entirely inconclusive (no driver in either tumor or a shared common driver, eg, *KRAS* G12C).

This implies that if tumor content is carefully confirmed, driver-only panels can distinguish between SPLCs and IPMs with fair confidence at a relatively high frequency (up to 80% of cases). However, it also underscores the need for more comprehensive panels in a subset of cases.

The diagrams for multiple NSCLC assessments by driver-only methods vs broad-panel NGS are summarized in Figure 1.[13]

## Major Lessons for Histologic Assessment of Multiple Non–Small Cell Lung Carcinomas

Currently, comprehensive histologic assessment is the main method for distinguishing SPLCs from IPMs in the AJCC staging manual. However, as mentioned above, recent studies using molecular methods have highlighted that although histologic assessment of adenocarcinoma relatedness is accurate in the majority of cases, a significant subset of cases may be misclassified based on histologic features alone.[9–17] For pulmonary squamous cell carcinoma, recent broad-panel NGS-based studies have demonstrated that histologic features are largely unreliable for

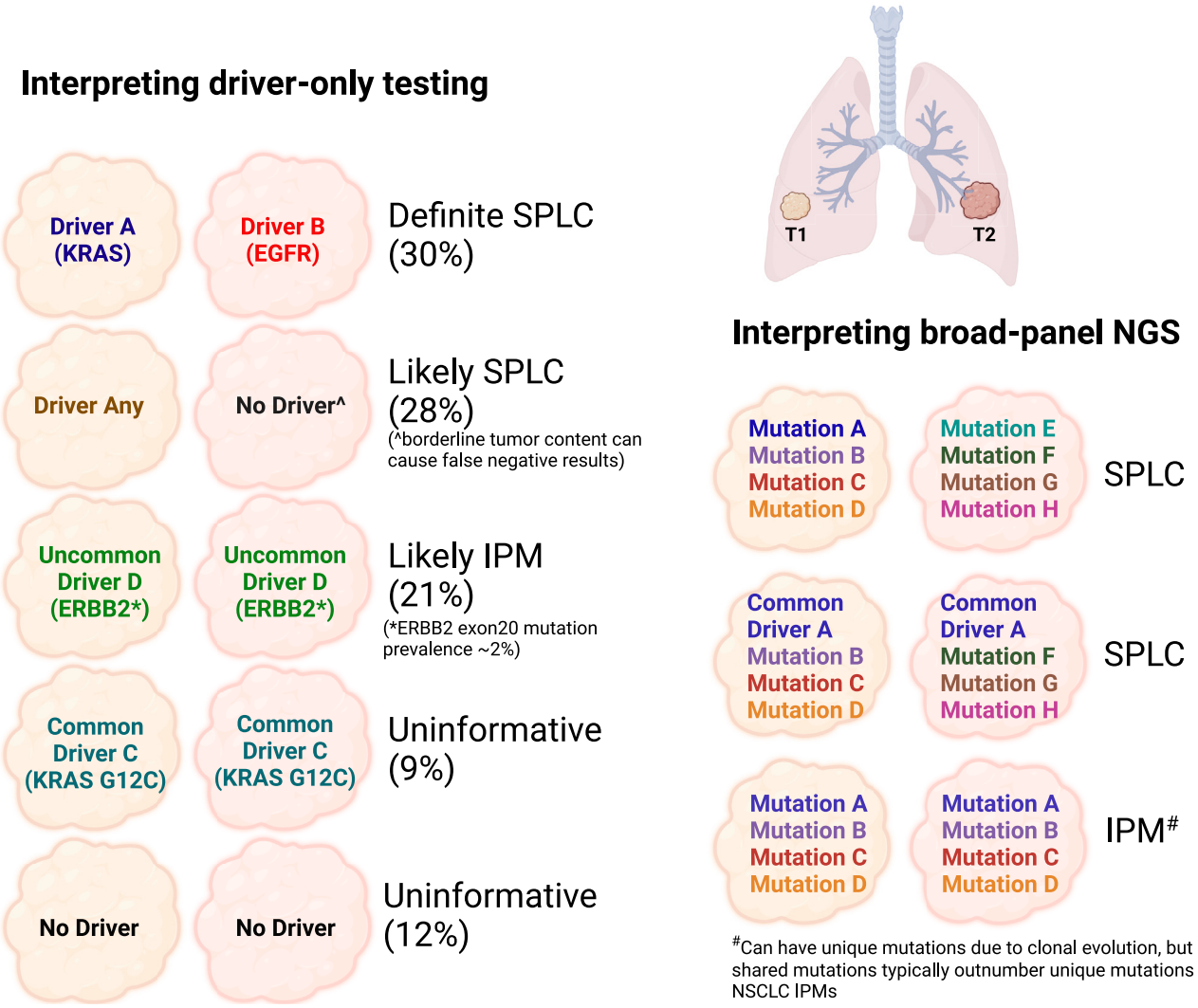



**Figure 1.**
Molecular profile interpretation in the evaluation of multiple non–small cell lung carcinomas using driver-only testing vs broad-panel NGS testing. Percentages are derived from Chang et al.[13] IPM, intrapulmonary metastasis; NGS, next-generation sequencing; NSCLC, non–small cell lung carcinomas; SPLC, separate primary lung cancer. Created with BioRender.com.

determining the relationship between multiple tumors with this histotype.[13,35]

Specific histology lessons are starting to emerge from studies using molecular methods as a gold standard:

(1) *Intrapulmonary metastases can be associated with histologic progression resulting in dissimilar histology.*

During clonal evolution, lung adenocarcinomas may acquire higher proportions of high-grade patterns, such as solid and micropapillary architectures.[41] Therefore, secondary lung tumors that appear morphologically dissimilar can still represent IPMs. Histologic progression rendering IPMs histologically different has been noted in 18% and 28% of cases in the studies by Yang et al[17] and Chang et al,[13] respectively.

Similarly, in squamous cell carcinoma, histologic features, such as the degree of keratinization and even basaloid cytology may not accurately differentiate between IPMs from SPLCs. Earlier studies have shown that squamous IPMs can exhibit a wide range of cytologic features and keratinization, making it difficult to make histologic predictions based on these characteristics alone.[35]

(2) *Intrapulmonary metastases can have a partial lepidic growth pattern.*

Lesions that appear radiologically as ground-glass opacities (GGO) or mixed GGOs typically correspond to tumors with predominant lepidic growth patterns on histology, including lepidic-predominant adenocarcinoma, minimally invasive adenocarcinoma (MIA), adenocarcinoma in situ (AIS), and atypical adenomatous hyperplasia (AAH). Such lesions generally represent SPLCs.[11] Multifocal lepidic-type GGO/mixed GGO lesions are typically encountered in current/former smokers in Western countries, but they may also occur in never-smokers in Asian countries.[42]

Remarkably, recent NGS studies have revealed that lepidic growth patterns can also occur in IPMs, likely because of tumor cells colonizing alveolar walls. This phenomenon is analogous to lung involvement by invasive mucinous adenocarcinoma (IMA) or

metastatic pancreatic adenocarcinoma, which tends to propagate along alveolar walls in a lepidic fashion. This fact challenges the assumption that lepidic pattern is invariably indicative of "in situ" growth, as it can also clearly represent colonization by invasive disease. The recent multiregion NGS study (TRACERx) identified genomic differences between lepidic patterns in early precursor lesions, such as AAH and AIS, and lepidic patterns in association with invasive carcinomas, supporting that lepidic patterns may not be synonymous with pre-invasive disease.[43] In the studies by Yang et al[17] and Chang et al,[13] a minor lepidic component was seen in 14% and 61% of tumor pairs, respectively, that were molecularly proven to be IPMs. In IPM cases studied to date, the lepidic component usually represented a minor portion of the tumor, although in some cases it was significant (up to 40%), with an even more extensive lepidic component recently reported.[13,43] Further study is needed to clarify the morphologic features in such unusual cases. Overall, multifocal lepidic-predominant adenocarcinoma, MIA, and AIS can still be regarded as SPLCs, whereas the presence of a minor lepidic component should not be considered as evidence of SPLCs.

(3) *Some similar-appearing lung adenocarcinomas may represent unrelated primaries.*

Lung adenocarcinomas exhibit extraordinary inherent histologic heterogeneity, with each tumor having a nearly unique combination of architectural patterns (such as acinar and papillary), cytologic features (such as mucinous features and clear cell features), stromal characteristics, and inflammatory milieu. However, despite this, certain morphologic features are extremely common among lung adenocarcinomas, and recent molecular studies have illustrated scenarios where resected adenocarcinomas appear similar but actually represent SPLCs. In retrospect, some differences can be appreciated, but this false similarity is a limitation for histologic assessment prospectively. In the MSKCC study, 12% of SPLCs were misclassified as IPMs due to false similarity.[13] A recent study by Bruehl et al[16] showed that overlapping architectural patterns alone may not be sufficiently specific for IPMs.[16] Therefore, it is imperative to take into account the cytologic features in the assessment of morphologic similarities; however, confidently separating similar-appearing SPLCs may not be feasible in all cases in practice.

An additional consideration is that adenocarcinoma with IPMs generally have at least focal micropapillary or solid patterns, and they tend to exhibit evidence of lymphovascular invasion and/or spread through airspaces (STAS). In the absence of any of these features, the interpretation of morphologically similar tumors as IPMs should be carefully re-evaluated.

Examples of straightforward cases and challenges in morphologic assessment of multiple NSCLCs are illustrated in Figures 2 to 6.

## Major Lessons for the Clinical Presentation of Separate Primary Lung Carcinomas vs Intrapulmonary Metastases

Recent NGS studies have challenged the robustness of several clinical parameters (such as latency, localization, and presence of nodal metastasis) in classifying multifocal NSCLCs, and highlighted previously underappreciated differences in patient populations for SPLCs and IPMs.

(1) *Molecular studies have revealed distinct patient populations for SPLCs and IPMs.* Studies based on the Western patient populations showed striking differences in several clinicopathologic parameters for patients with SPLCs vs IPMs.[10,13] First, patients with SPLCs were almost entirely current/former smokers, whereas patients with IPMs had variable smoking histories. Second, SPLCs were dominated by *KRAS* mutations, consistent with the smoking history, whereas IPMs were enriched in *EGFR* and *MET* exon 14 mutations. The differences in patient populations and underlying genomic findings highlight the distinct biology underlying the pathogenesis of SPLCs and IPMs (see next section). As mentioned above, in Asian countries, patients with SPLCs are commonly never-smokers with a predominance of *EGFR* mutations, so the differences in patient characteristics for SPLCs vs IPMs may not apply.[17]

(2) *Molecular studies have shown that IPMs can occur after a significantly longer period than 2 years.* The original Martini and Melamed/ACCP criteria for metachronous SPLCs vs IPMs include the time period between the surgery and the appearance of the second tumor, with over 2 years favoring a new primary over IPMs. However, NGS-based studies have confirmed the possibility of late recurrences after surgery, where the lungs may be the only site of recurrent disease. In the MSKCC series of nonmucinous adenocarcinomas, NGS confirmed multiple cases of IPMs more than 2 years (up to 7.6 years) following the initial tumor resection.[13] As discussed below, molecular studies also confirm the possibility of even more prolonged latency to recurrence for mucinous lung adenocarcinomas.

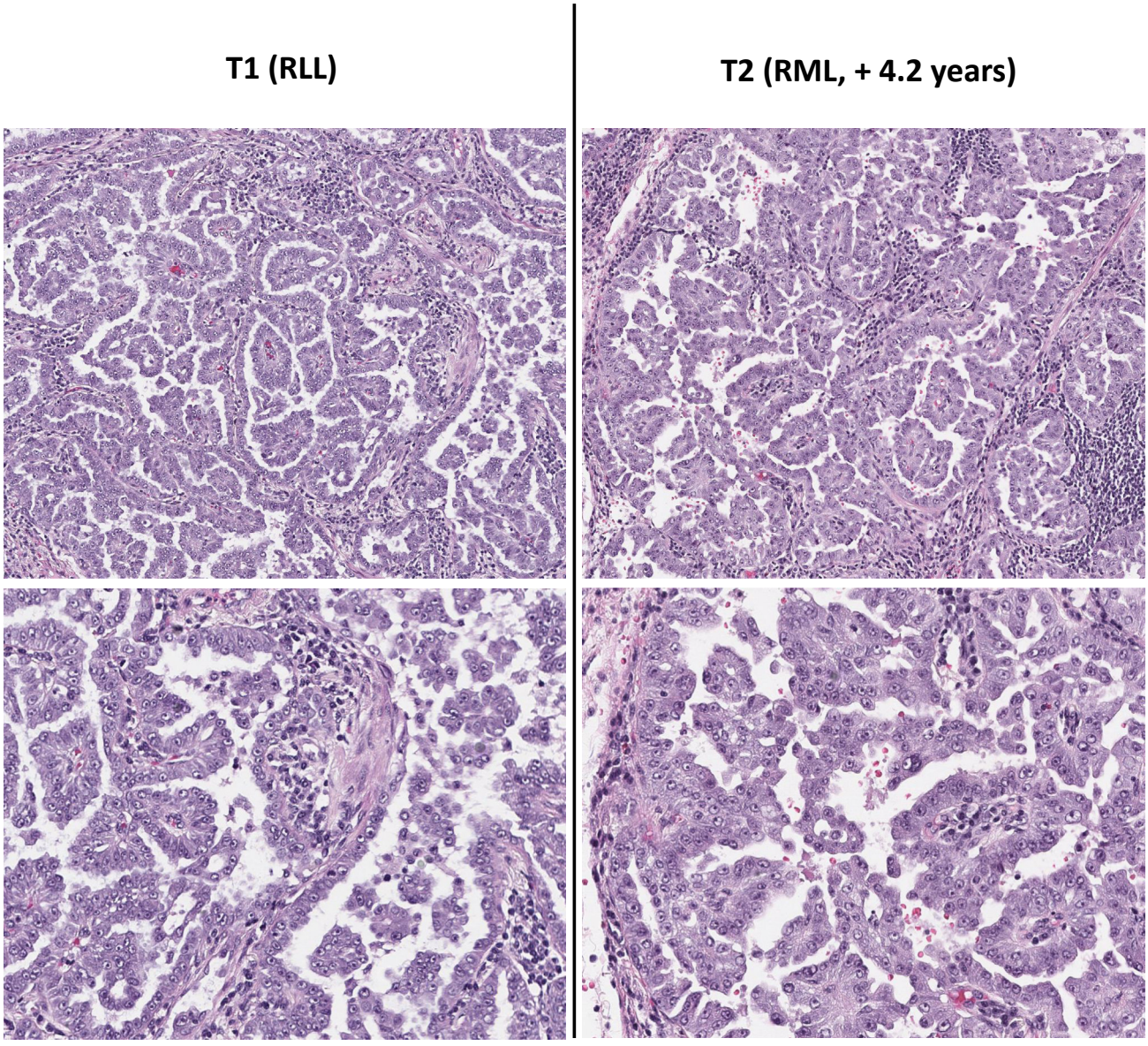
(3) *Molecular studies have established that IPMs can involve the contralateral lung without ipsilateral lesions.* Although the Martini and Melamed criteria suggested that IPMs are more prone to occur in the same lobe, subsequent studies have not clearly demonstrated spatial exclusivity for IPMs.[10,13,17] In particular, in the MSKCC series, some patients developed contralateral IPMs without lesions in the same lobe.[13]

(4) *Molecular studies have established that IPMs may occur in the absence of nodal and distant metastases.* The Martini and Melamed criteria and ACCP guidelines consider the presence of regional lymph node involvement and distant metastasis to favor IPMs. The predictive value of these variables has not been substantiated by the recent study by our group, in which most IPMs lacked lymph node involvement, suggesting that the presence of lymphatic involvement is not a prerequisite for tumor spread across different lobes, and also highlighting likely unique pathophysiologic mechanisms of tumor intrapulmonary spread.[13]

These emerging findings suggest that a major reassessment of the clinical criteria used to distinguish SPLCs from IPMs is needed. Although certain clinical parameters may favor SPLCs vs IPMs, molecular data clearly indicate that none are definitive and there are common exceptions. In particular, because IPMs may present as an isolated lesion in a different lobe without nodal/distant metastasis after a >2-year period, these are commonly suspected to represent new primary tumors on clinical grounds.

## Implications for the Understanding of the Biology of Intrapulmonary Spread and Separate Primary Lung Carcinomas

With a robust molecular gold standard, studies are starting to shed light on the underlying biology of intrapulmonary spread

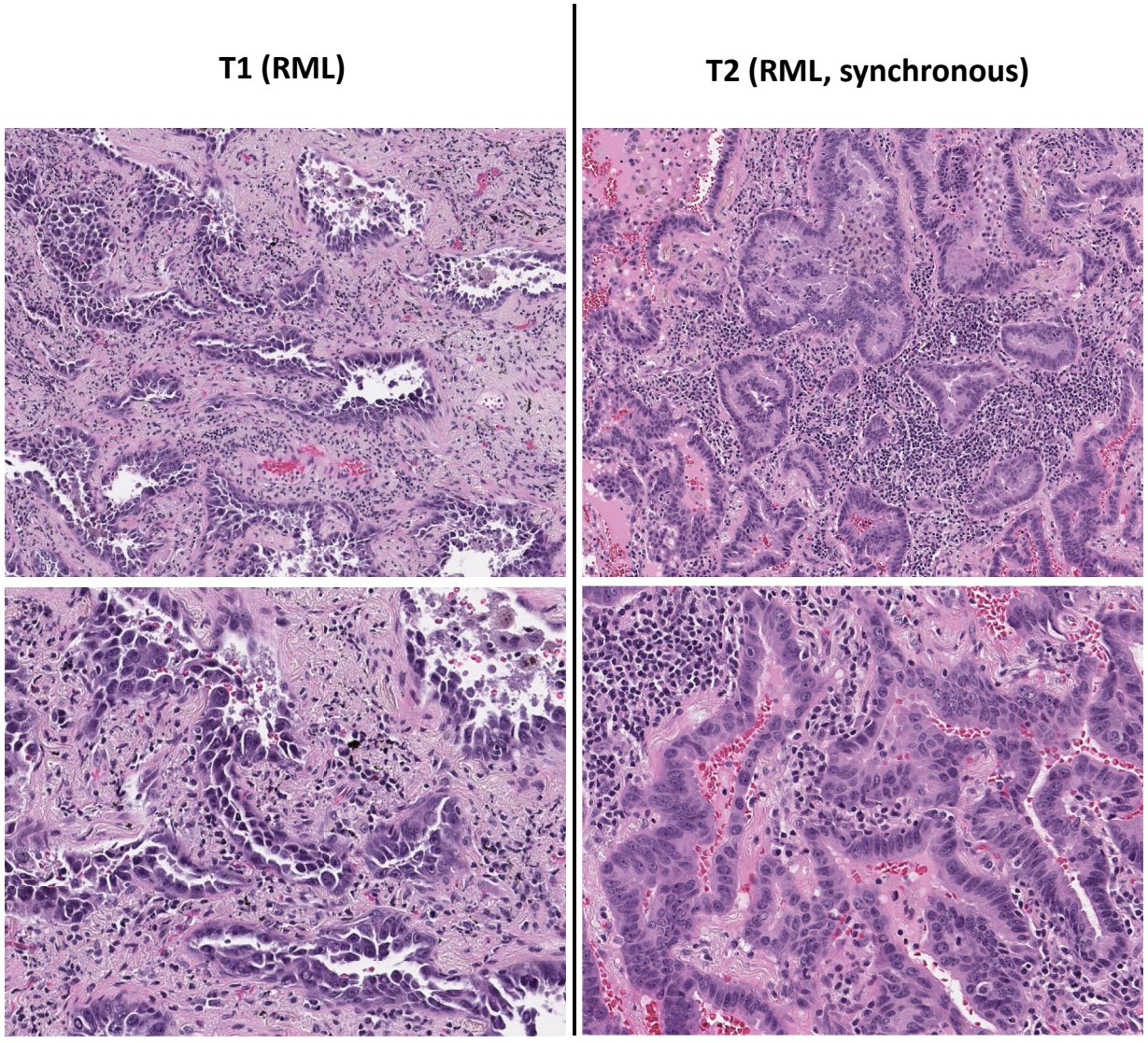



| | T1 | T2 |
|---|---|---|
| CD74-ROS1 fusion | + | + |
| AXIN1 exon2 p.Y237* (c.711C>A) | + | + |
| CTNNB1 exon3 p.D32G (c.95A>G) | + | + |
| DNMT3A exon12 splicing variant p.X492_splice (c.1474+1G>A) | + | + |
| DNMT3A exon19 p.R749C (c.2245C>T) | + | |
| RB1 exon17 splicing variant p.X500_splice (c.1499-1G>A) | | + |

**Figure 2.**
Histologic appearance and next-generation sequencing profile of an intrapulmonary metastasis (IPM) showing overlapping morphologic features and genomic profiles. The presence of unique mutations in each of the tumors reflects clonal evolution and does not interfere with the interpretation of clonal relatedness given multiple shared mutations. RLL, right lower lobe; RML, right middle lobe.

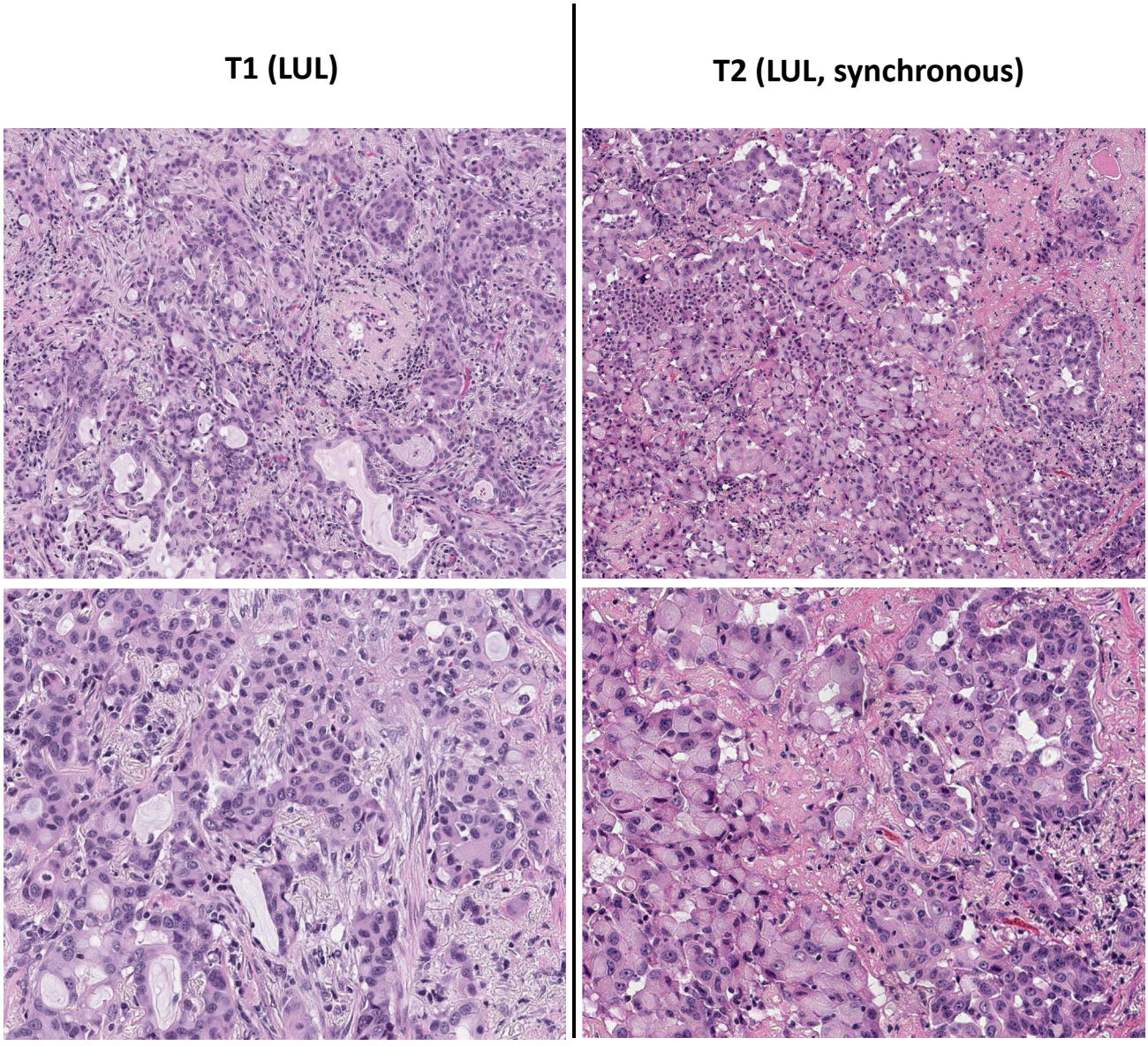
(IPM) and multifocal NSCLC (SPLCs) carcinogenesis and provide answers to previously unsolved questions in this field.

For IPMs, an important issue in surgical series is that it generally represents resectable oligometastatic disease, where patients present with only 1 to 2 metastatic lesions involving the lung and no evidence of extrapulmonary disease. Although extensive IPM is a well-recognized disease radiologically and pathologically, oligometastatic IPM is still understudied in part, because definitive diagnosis may require robust molecular confirmation.




| | T1 | T2 |
|---|---|---|
| KRAS exon2 p.G13C (c.37G>T) | + | |
| TP53 exon5 splicing variant p.X187_splice (c.559+1G>A) | + | |
| ATRX exon9 splicing variant p.X221_splice (c.663-1G>T) | + | |
| BTK exon18 p.G594W (c.1780G>T) | + | |
| ERG exon7 p.P254Tfs*37 (c.760_761delinsA) | + | |
| TCF3 exon2 splicing variant p.X25_splice (c.73-1G>C) | + | |
| KRAS exon2 p.G12D (c.35G>A) | | + |
| MSI2 exon6 p.K135Q (c.403A>C) | | + |
| PPM1D exon1 p.S44W (c.131C>G) | | + |
| PTPRD exon28 p.G855C (c.2563G>T) | | + |
| RBM10 exon10 p.G409C (c.1225G>T) | | + |

**Figure 3.**
Histologic appearance and next-generation sequencing profile of separate primary lung carcinomas (SPLCs) showing distinct morphologic features and genomic profiles. RML, right middle lobe.

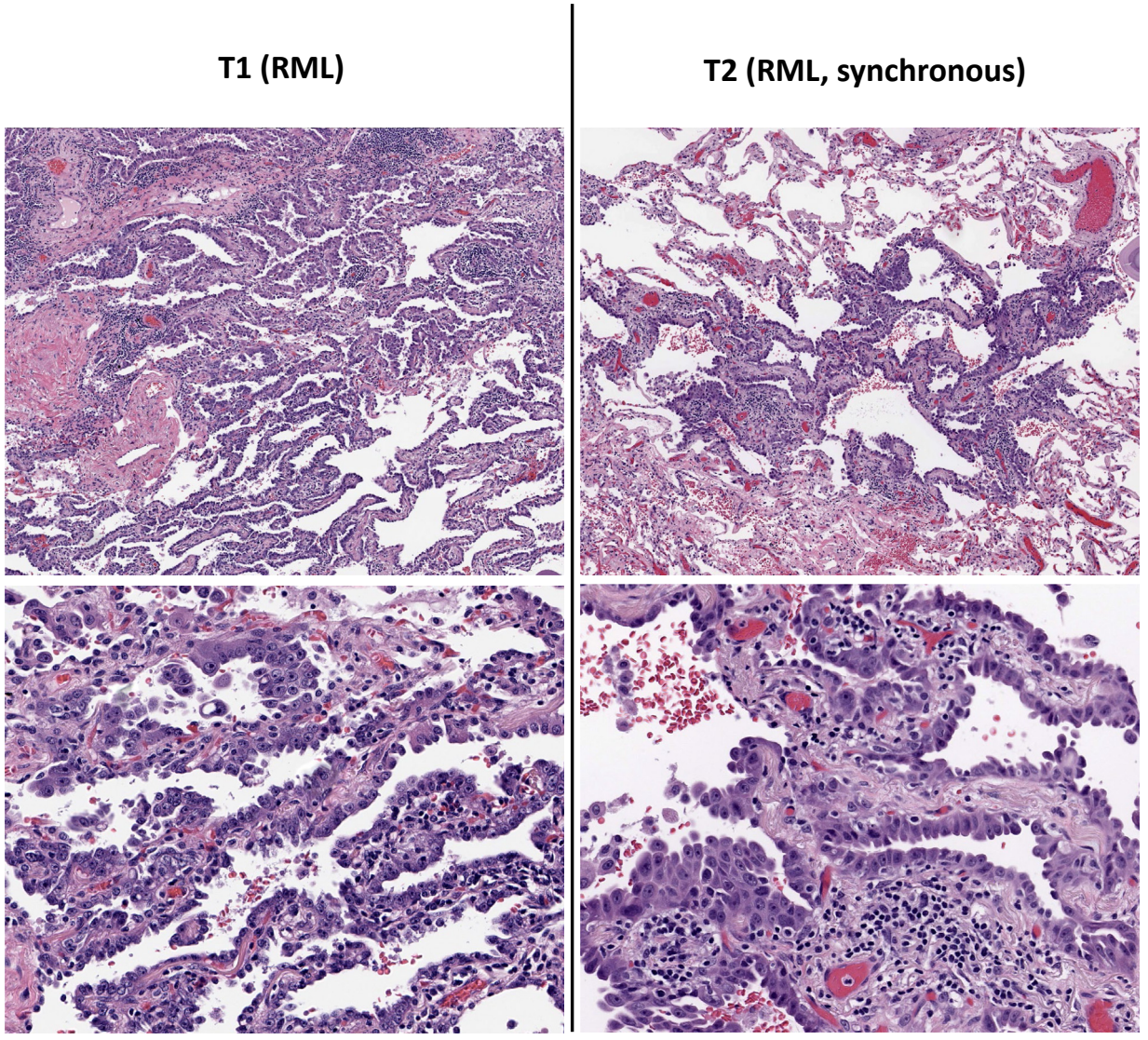



| | T1 | T2 |
|---|---|---|
| EGFR exon20 p.H773_V774insAH (c.2315_2320dupCCCACG) | + | + |
| GLI1 exon12 p.R648H (c.1943G>A) | + | + |
| HIST1H3I (c.*55C>A) | + | + |
| STAG2 (c.2534-175T>C) | + | + |

**Figure 4.**
Histologic appearance and next-generation sequencing profile of an intrapulmonary metastasis (IPM) showing the histologic progression from acinar/cribriform patterns in the primary tumor (T1, left) to solid patterns with extensive signet-ring cell features in the metastasis (T2, right). Despite architectural evolution, the cytologic features remain similar; however, definitive assessment based on morphology alone may be challenging. LUL, left upper lobe.

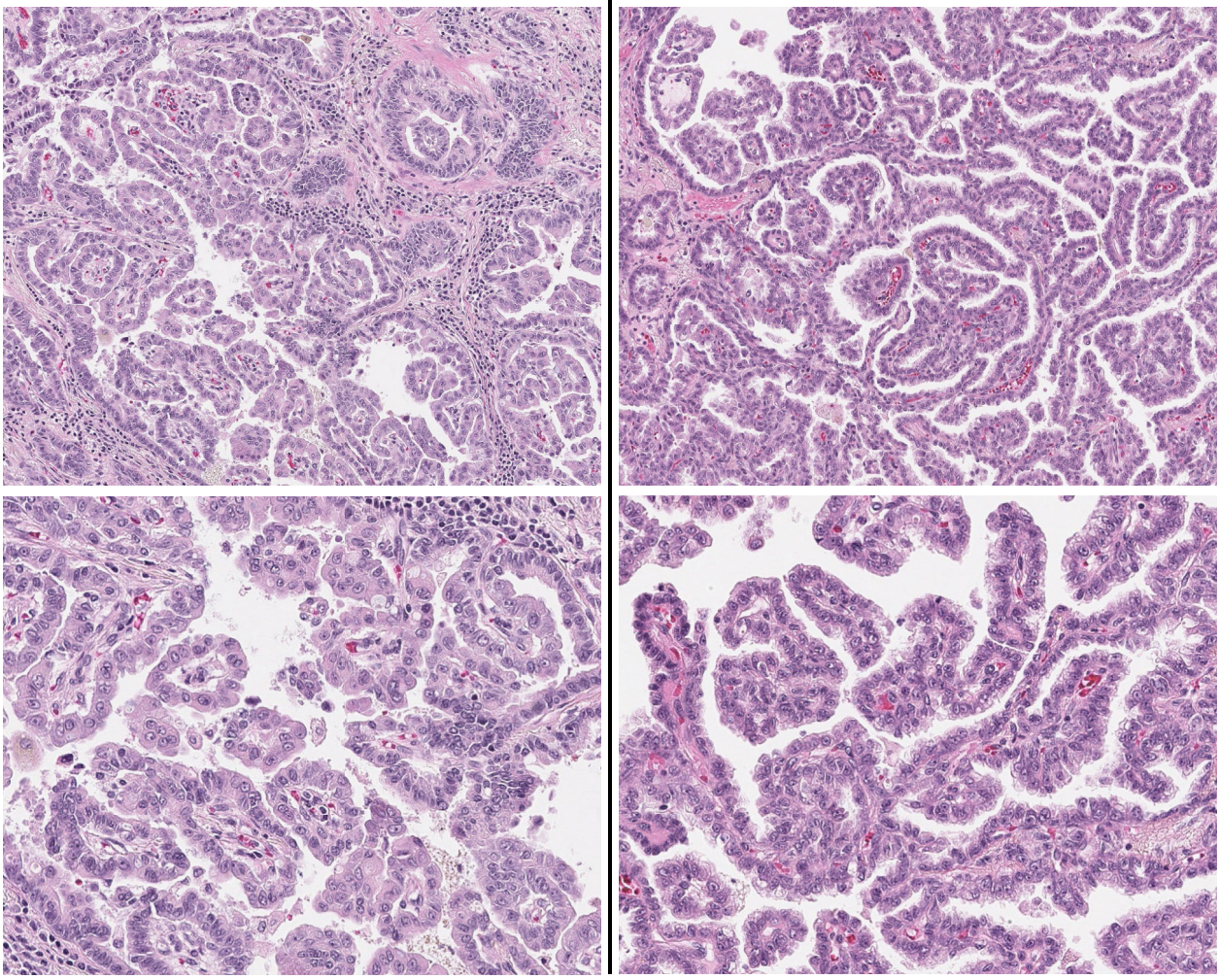
The challenge of accurate pathologic staging of IPMs pertains specifically to this subset of patients whose tumors spread, at least initially, in a localized manner to isolated lung location(s). The underlying pathogenesis of oligometastatic IPMs, and whether it occurs through pulmonary lymphovascular spread or STAS, remains uncertain. Additionally, the reason for the high prevalence of non–smoking-related driver alterations, such as *EGFR* and *MET* mutations, in IPMs is unclear but may be related to the unique biology of tumors harboring these alterations.

In contrast, the strong association between smoking and SPLCs highlights the clear role of smoking-related lung damage as a predisposing factor for the development of independent multifocal lung cancers in Western populations. The enrichment in *KRAS* G12C (and other transversion *KRAS* mutations) in SPLCs supports the established link between smoking and multifocal carcinogenesis.




| | T1 | T2 |
|---|---|---|
| KRAS exon2 p.G12D (c.35G>A) | + | + |
| PIK3CA exon10 p.E545K (c.1633G>A) | + | + |
| CCND2 exon5 p.T282K (c.845C>A) | + | + |
| CTLA4 exon2 p.E94K (c.280G>A) | + | + |
| EIF1AX exon2 p.R13S (c.37C>A) | + | + |
| ERBB4 exon9 p.V348M (c.1042G>A) | + | + |
| NFE2L2 exon2 p.E45K (c.133G>A) | + | + |
| SETD2 exon13 splicing variant p.X2021_splice (c.6061-1G>T) | + | + |
| SPEN exon11 p.Q3342* (c.10024C>T) | + | + |
| ARID1A exon18 p.Q1479* (c.4435C>T) | + | |
| ARAF exon8 p.D242N (c.724G>A) | | + |

**Figure 5.**
Histologic appearance and next-generation sequencing profile of an intrapulmonary metastasis (IPM) with lepidic patterns observed in both the primary tumor (T1, left; 30% lepidic) and the metastasis (T2, right; 20% lepidic). Although the high-power photomicrograph of the metastasis only illustrates the lepidic component, invasive patterns are present in other parts of the tumor. Notably, the cytologic features of the lepidic component exhibit greater atypia than typically seen in precursor lesions, such as adenocarcinoma in situ/minimally invasive adenocarcinoma, and instead more closely resemble those of the invasive component. RML, right middle lobe.




| | T1 | T2 |
|---|---|---|
| KRAS exon2 p.Q22K (c.64C>A) | + | |
| KEAP1 exon3 p.R413C (c.1237C>T) | + | |
| MED12 exon15 p.D705Y (c.2113G>T) | + | |
| PIK3C2G exon2 p.G210V (c.629G>T) | + | |
| STK11 exon1 p.K44Sfs*7 (c.131delA) | + | |
| BRAF exon11 p.G466V (c.1397G>T) | | + |
| KEAP1 exon6 p.M597Rfs*75 (c.1790_1797delinsGGCCTTC) | | + |
| PBRM1 exon22 p.E1107Rfs*11 (c.3318dupA) | | + |
| RBM10 exon23 p.Q915* (c.2743C>T) | | + |
| STK11 exon4 p.Q159_I161delinsH (c.477_482delGCTGAT) | | + |

**Figure 6.**
Histologic appearance and next-generation sequencing profile of separate primary lung carcinomas (SPLCs) showing overlapping morphologic features characterized by extensive papillary and micropapillary patterns (false similarity) despite entirely distinct genomic profiles. Subtle differences in cytologic features, such as the presence of densely eosinophilic cytoplasm in T1 vs clear cell changes in T2, may suggest that these tumors are not the same; however, definitive determination of tumor relationship would be difficult by morphology alone. LUL, left upper lobe.

An intriguing observation in several studies to date has been that the rate of single shared drivers in unrelated adenocarcinomas (SPLCs) is even higher than what would be predicted by chance alone.[13,17] This suggests that the odds of a particular mutation arising in an individual may be influenced by specific environmental or hereditary factors, in line with the concept of convergent evolution.[44]

The risk factors for SPLCs in never-smokers are a particularly enigmatic and poorly understood area. However, it is known that there are rare patients with germline weakly activating *EGFR* T790M mutations, and such patients are at a heightened risk of developing multiple SPLCs harboring additional somatic *EGFR* mutations.[45,46] Identification of germline predisposition mutations offers insight into the underlying biology in some patients with SPLCs, although presumably an extremely rare subset. Importantly, this further highlights the utility of paired tumor/normal sequencing in comparative molecular profiling.

## Emerging Clinical Significance

IPMs and SPLCs represent 2 entirely distinct biological processes. Generally, adjuvant systemic therapy may be considered in patients with confirmed IPMs, even if the tumors are fully resected. In contrast, management for SPLCs is based on the stage of the most advanced tumor. In the pre-NGS era, the efforts of establishing clear clinical guidelines for patients with IPMs and SPLCs were markedly hampered by the difficulties in accurately separating these 2 processes with a high number of unclassified or misclassified cases. However, recent NGS studies showed that patients with IPMs have significantly shorter survival or a trend toward worse survival compared with patients with multiple SPLCs.[12,13,17,47,48] Continued accumulation of data on the prognostic outcomes of multiple NSCLCs defined by robust molecular methods is needed.

With the increasing adoption of molecular techniques, enabling a higher level of accuracy in distinguishing between IPMs and SPLCs in practice, a substantial improvement in the understanding of the biology and clinical outcomes in these 2 distinct patient groups can be anticipated. This development is poised to significantly inform and refine clinical management practices.

## Emerging Lessons for the Radiologic Presentation of SPLCs vs IPMs Using Molecular Methods as a Gold Standard

Whether multiple lung cancers can be separated into SPLCs vs IPMs radiologically has been a long-standing question. Here too, the limitation in prior studies has been the lack of a robust gold standard. Recently, using tumors in which the clonal relationships were established using broad-panel NGS as the ground truth, Araujo-Filho et al[49] showed that imaging characteristics on CT have some distinct characteristics. Specifically, SPLCs were associated with subsolid consistency and spiculated contours, whereas IPMs were associated with greater size difference between the tumors and pure solid consistency in the smaller tumors. However, none of these radiologic features were entirely sensitive or specific, and therefore definitive assessment of clonal relationships generally requires pathologic/molecular assessment.

## Special Issues Related to the Assessment of Multiple NSCLCs

### *Invasive Mucinous Adenocarcinomas*

Invasive mucinous adenocarcinoma (IMA) is a distinct subtype of lung adenocarcinoma with a unique propensity for multifocal or multilobar distribution. Recent studies by Yang et al[50] and Kim et al[51] found that the vast majority of IMAs presenting with spatially separate lesions represented clonally related IPMs, including cases with the contralateral distribution. This is highly distinct from nonmucinous adenocarcinoma, in which most multifocal NSCLCs are SPLCs. One particularly noteworthy finding for metachronous IMA is the confirmation of ultralate recurrences, which were clinically considered as new primary tumors due to the extended latency period of up to 12 years between initial tumor resection and detection of a clonally related tumor, sometimes in a contralateral lobe.[50,52]

Although isolated cases of multifocal IMA were identified as clonally unrelated, generally, given the high probability of multifocal IMA lesions representing intrapulmonary spread, driver-only testing should be sufficient to support the clonal relationship of separate IMA nodules. Cases with “pneumonic”/consolidative involvement of one or multiple lobes consistently represented IPMs, and do not require molecular confirmation.

### *Multifocal Ground-glass/Lepidic Lesions*

The eighth edition of the AJCC Cancer Staging Manual provides guidelines for classifying tumors as multifocal ground-glass/lepidic adenocarcinoma.[18] This classification is based on clinical/radiologic findings of multiple subsolid nodules (either pure ground-glass or part solid), with at least one being suspected or proven to be neoplastic. Pathologically, the disease process corresponds to multiple, sometimes innumerable, foci of lepidic-predominant adenocarcinoma, MIA, and AIS. Such lesions represent SPLCs and do not require molecular testing for confirmation.[53-55]

### *The Feasibility of Comparative Profiling in Biopsies*

Histologic assessment of biopsy specimens can be particularly challenging due to limited tissue quantity. Despite the scarcity of data, our experience suggests that, in some cases, it is possible to suggest whether tumors are related or unrelated; however, in many biopsies, the morphologic comparison should only be considered provisional, until molecular results (or resection) become available.

## Approach for Current Practice: Histology vs Molecular – When and How?

1. *What are the scenarios where histologic assessment is sufficient?*
   - Lesions entirely or predominantly displaying lepidic patterns (lepidic-predominant adenocarcinoma, MIA, AIS) may be considered and staged as separate primaries without the need for molecular confirmation. Importantly, a minor lepidic component should not be regarded as evidence of “in situ” disease to support the interpretation of tumors as SPLCs.
   - Tumors with entirely nonoverlapping histology devoid of high-grade patterns (micropapillary, solid) can be accepted as SPLCs without molecular confirmation. However, it is crucial to exercise caution when evaluating tumors harboring high-grade patterns, even if these patterns represent only a minor component of the tumor.[13,43]
   - Tumors, where pathologists can make an assessment that the morphologic features are identical with high confidence, do not require molecular confirmation. Importantly,

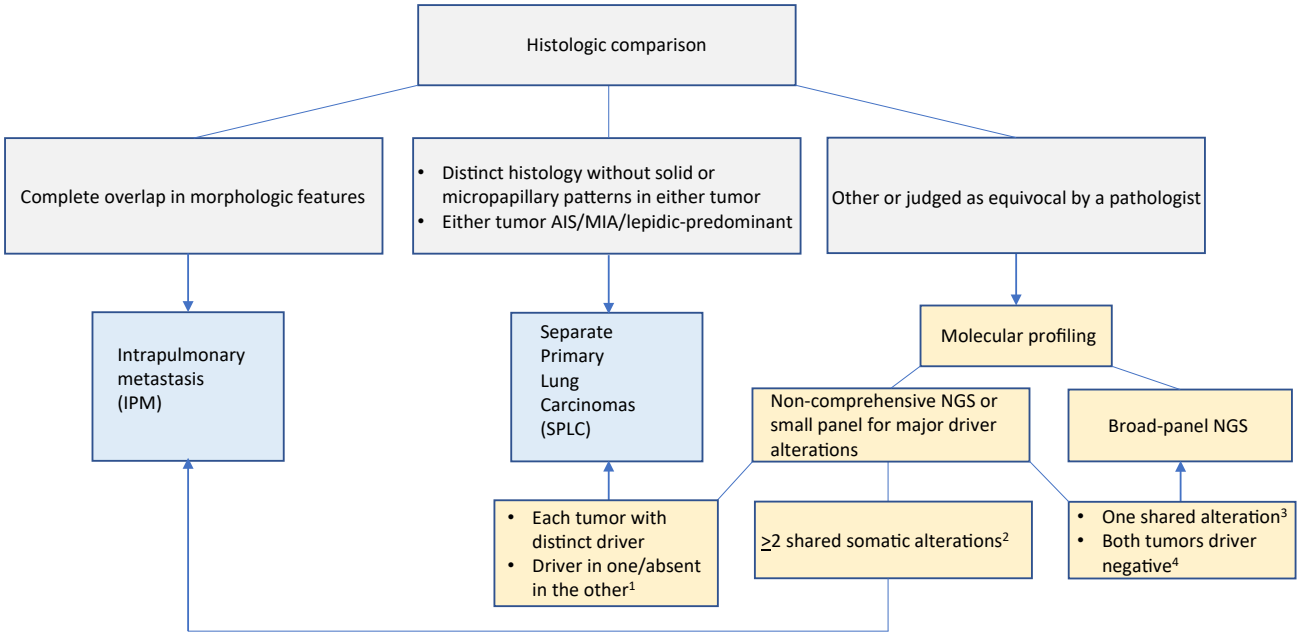



**Figure 7.**
Flowchart for classification of multiple NCSLC using histology and molecular results (reproduced with permission from Chang et al).[13] (1), This scenario supports SPLCs only if adequate tumor content has been confirmed histologically in the driver-negative tumor. (2) Applicable to NGS only but not major driver-only testing. (3) If only a single alteration is shared, the degree to which this supports IPMs should be determined on the basis of prevalence of that alteration in a given population as well as overall clinicoradiologic context. (4) Driver negative in both tumors would benefit from broad-panel NGS for comprehensive comparison of mutation profiles. AIS, adenocarcinoma in situ; MIA, minimally invasive adenocarcinoma; NGS, next-generation sequencing; SPLC, separate primary lung carcinoma.

morphologic assessment should encompass not only architectural patterns but also a comparison of cytologic features, stromal characteristics, as well as the extent and type of associated inflammation. As tumors with IPMs typically display at least focal micropapillary/solid patterns, STAS, and/or lymphovascular invasion, the complete absence of all these features would argue against IPMs even for similar-appearing tumors.

2. *What molecular methods should be utilized?* As discussed above, the majority of multiple NSCLCs can be classified as separate vs same tumors with definite or likely interpretation using driver gene-only approach. However, in scenarios where driver-only testing is uninformative or more definite interpretation is desired, NGS should be considered. If unavailable, staging should be based on the pathologic comparison of tumors in the context of the available clinical and radiologic information. The clinical modifiers that have distinct associations with SPLCs vs IPMs (such as the link between SPLCs with smoking vs IPMs with never-smoking history in Western patients, and other clinicopathologic parameters discussed above) may aid in the assessment.

The stepwise approach for multiple NSCLC assessment using morphologic evaluation and different types of molecular studies is outlined in Figure 7.[13]

## Staging of Multiple NSCLCs: Should Staging be Assigned Before or After Molecular Studies?

Recent molecular studies have highlighted the limitations of multiple NSCLC assessment based on clinical and morphologic parameters alone, underscoring the necessity for comparative molecular profiling in a subset of cases for accurate staging, and raising an unprecedented issue for staging in lung cancer. Although a preliminary histologic impression may be feasible in most cases, there is a subset of cases where an accurate stage cannot be determined at the time of pathologic evaluation.

Given that molecular sequencing results require additional days or weeks, depending on the specific assays used, the final staging that integrates both histologic and molecular data may not be feasible at the time of the initial surgical pathology report. To address this, one approach would be to assign a provisional stage, subject to amendment based on subsequent molecular findings. Alternatively, staging could be postponed until comparative molecular profiling is completed. Currently, the College of American Pathologists templates mandate the inclusion of pathologic stage in lung cancer resection reports. However, consideration should be given to a stepwise approach, where if indicated, final staging is deferred until molecular results are available.

## Conclusion

In summary, the approach to evaluating multiple NSCLCs is undergoing a paradigm shift, with increasing emphasis on supplementing pathologic assessment with comparative molecular testing. This change marks a critical development in pathology practice as it significantly enhances the accuracy of differentiating between 2 biological processes that may overlap in clinicopathologic presentation yet are inherently distinct. The implications of this paradigm shift are expected to extend beyond diagnostic accuracy but also influence the staging process, which is pivotal in formulating effective clinical management strategies and accurately prognosticating survival outcomes. Consequently, it is anticipated that future staging guidelines will require substantial updates, highlighting the need for continuous adaptation and refinement to incorporate these molecular techniques.

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*Data Availability*

Not applicable.

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*Declaration of Competing Interest*

The authors declare no conflicts of interest.

## Supplementary Material

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## References

1. Flieder DB, Vazquez M, Carter D, et al. Pathologic findings of lung tumors diagnosed on baseline CT screening. *Am J Surg Pathol*. 2006;30:606–613. https://doi.org/10.1097/01.pas.0000202040.51967.d0
2. Vazquez M, Carter D, Brambilla E, et al. Solitary and multiple resected adenocarcinomas after CT screening for lung cancer: histopathologic features and their prognostic implications. *Lung Cancer*. 2009;64:148–154. https://doi.org/10.1016/j.lungcan.2008.08.009
3. Lou F, Huang J, Sima CS, Dycoco J, Rusch V, Bach PB. Patterns of recurrence and second primary lung cancer in early-stage lung cancer survivors followed with routine computed tomography surveillance. *J Thorac Cardiovasc Surg*. 2013;145:75–81. https://doi.org/10.1016/j.jtcvs.2012.09.030
4. Mascalchi M, Comin CE, Bertelli E, et al. Screen-detected multiple primary lung cancers in the ITALUNG trial. *J Thorac Dis*. 2018;10:1058–1066. https://doi.org/10.21037/jtd.2018.01.95
5. Martini N, Melamed MR. Multiple primary lung cancers. *J Thorac Cardiovasc Surg*. 1975;70:606–612. https://doi.org/10.1016/S0022-5223(19)40289-4
6. Kozower BD, Larner JM, Detterbeck FC, Jones DR. Special treatment issues in non-small cell lung cancer diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. *Chest*. 2013;143:e369S–e399S. https://doi.org/10.1378/chest.12-2362
7. Girard N, Deshpande C, Lau C, et al. Comprehensive histologic assessment helps to differentiate multiple lung primary nonsmall cell carcinomas from metastases. *Am J Surg Pathology*. 2009;33:1752–1764. https://doi.org/10.1097/PAS.0b013e3181b8cf03
8. Takamochi K, Oh S, Matsuoka J, Suzuki K. Clonality status of multifocal lung adenocarcinomas based on the mutation patterns of EGFR and K-ras. *Lung Cancer*. 2012;75:313–320. https://doi.org/10.1016/j.lungcan.2011.08.007
9. Schneider F, Derrick V, Davison JM, Strollo D, Incharoen P, Dacic S. Morphological and molecular approach to synchronous non-small cell lung carcinomas: impact on staging. *Mod Pathol*. 2016;29:735–742. https://doi.org/10.1038/modpathol.2016.66
10. Mansuet-Lupo A, Barritault M, Alifano M, et al. Proposal for a combined histomolecular algorithm to distinguish multiple primary adenocarcinomas from intrapulmonary metastasis in patients with multiple lung tumors. *J Thorac Oncol*. 2019;14:844–856. https://doi.org/10.1016/j.jtho.2019.01.017
11. Asmar R, Sonett JR, Singh G, Mansukhani MM, Borczuk AC. Use of oncogenic driver mutations in staging of multiple primary lung carcinomas: a single-center experience. *J Thorac Oncol*. 2017;12:1524–1535. https://doi.org/10.1016/j.jtho.2017.06.012
12. Roepman P, Heuvel At, Scheidel KC, et al. Added value of 50-gene panel sequencing to distinguish multiple primary lung cancers from pulmonary metastases: a systematic investigation. *J Mol Diagn*. 2018;20:436–445. https://doi.org/10.1016/j.jmoldx.2018.02.007
13. Chang JC, Alex D, Bott M, et al. Comprehensive next-generation sequencing unambiguously distinguishes separate primary lung carcinomas from intrapulmonary metastases: comparison with standard histopathologic approach. *Clin Cancer Res*. 2019;25:7113–7125. https://doi.org/10.1158/1078-0432.CCR-19-1700
14. Higuchi R, Nakagomi T, Goto T, et al. Identification of clonality through genomic profile analysis in multiple lung cancers. *J Clin Med*. 2020;9:573. https://doi.org/10.3390/jcm9020573
15. Pagan CA, Shu CA, Crapanzano JP, et al. Synchronous pulmonary adenocarcinomas. *Am J Clin Pathol*. 2020;154:57–69. https://doi.org/10.1093/ajcp/aqaa023
16. Bruehl FK, Doxtader EE, Cheng Y-W, Farkas DH, Farver C, Mukhopadhyay S. Does histological assessment accurately distinguish separate primary lung adenocarcinomas from intrapulmonary metastases? A study of paired resected lung nodules in 32 patients using a routine next-generation sequencing panel for driver mutations. *J Clin Pathol*. 2021;75(6):390–396. https://doi.org/10.1136/jclinpath-2021-207421jclinpath-2021-207421
17. Yang CY, Yeh YC, Wang LC, et al. Genomic profiling with large-scale next-generation sequencing panels distinguishes separate primary lung adenocarcinomas from intrapulmonary metastases. *Mod Pathol*. 2023;36:100047. https://doi.org/10.1016/j.modpat.2022.100047
18. Amin MB, Edge SB, Greene F, et al. *AJCC Cancer Staging Manual*. 8th ed. Springer International Publishing; 2017.
19. Murphy SJ, Harris FR, Kosari F, et al. Using genomics to differentiate multiple primaries from metastatic lung cancer. *J Thorac Oncol*. 2019;14:1567–1582. https://doi.org/10.1016/j.jtho.2019.05.008
20. Huang J, Behrens C, Wistuba I, Gazdar AF, Jagirdar J. Molecular analysis of synchronous and metachronous tumors of the lung: impact on management and prognosis. *Ann Diagn Pathol*. 2001;5:321–329. https://doi.org/10.1053/adpa.2001.29338
21. Shen C, Wang X, Tian L, Che G. Microsatellite alteration in multiple primary lung cancer. *J Thorac Dis*. 2014;6:1499–1505. https://doi.org/10.3978/j.issn.2072-1439.2014.09.14
22. Shimizu S, Yatabe Y, Koshikawa T, et al. High frequency of clonally related tumors in cases of multiple synchronous lung cancers as revealed by molecular diagnosis. *Clin Cancer Res*. 2000;6:3994–3999. PMID:11051248.
23. Wang X, Wang M, MacLennan GT, et al. Evidence for common clonal origin of multifocal lung cancers. *J Natl Cancer Inst*. 2009;101:560–570. https://doi.org/10.1093/jnci/djp054
24. Arai J, Tsuchiya T, Oikawa M, et al. Clinical and molecular analysis of synchronous double lung cancers. *Lung Cancer*. 2012;77:281–287. https://doi.org/10.1016/j.lungcan.2012.04.003
25. Mitsudomi T, Yatabe Y, Koshikawa T, et al. Mutations of the p53 tumor suppressor gene as clonal marker for multiple primary lung cancers. *J Thorac Cardiovasc Surg*. 1997;114:354–360. https://doi.org/10.1016/S0022-5223(97)70180-6
26. van Rens MTM, Eijken EJE, Elbers JRJ, Lammers JWJ, Tilanus MGJ, Slootweg PJ. p53 mutation analysis for definite diagnosis of multiple primary lung carcinoma. *Cancer*. 2002;94:188–196. https://doi.org/10.1002/cncr.10001
27. Warth A, Macher-Goeppinger S, Muley T, et al. Clonality of multifocal non-small cell lung cancer: implications for staging and therapy. *Eur Respir J*. 2012;39:1437–1442. https://doi.org/10.1183/09031936.00105911
28. Wu C, Zhao C, Yang Y, et al. High discrepancy of driver mutations in patients with NSCLC and synchronous multiple lung ground-glass nodules. *J Thorac Oncol*. 2015;10:778–783. https://doi.org/10.1097/JTO.0000000000000487
29. de Bruin EC, McGranahan N, Mitter R, et al. Spatial and temporal diversity in genomic instability processes defines lung cancer evolution. *Science*. 2014;346:251–256. https://doi.org/10.1126/science.1253462
30. Saab J, Zia H, Mathew S, Kluk M, Narula N, Fernandes H. Utility of genomic analysis in differentiating synchronous and metachronous lung adenocarcinomas from primary adenocarcinomas with intrapulmonary metastasis. *Transl Oncol*. 2017;10:442–449. https://doi.org/10.1016/j.tranon.2017.02.009
31. Patel SB, Kadi W, Walts AE, et al. Next-generation sequencing: a novel approach to distinguish multifocal primary lung adenocarcinomas from intrapulmonary metastases. *J Mol Diagn*. 2017;19:870–880. https://doi.org/10.1016/j.jmoldx.2017.07.006
32. Takahashi Y, Shien K, Tomida S, et al. Comparative mutational evaluation of multiple lung cancers by multiplex oncogene mutation analysis. *Cancer Sci*. 2018;109:3634–3642. https://doi.org/10.1111/cas.13797
33. Liu J, Mao G, Li Y, et al. Targeted deep sequencing helps distinguish independent primary tumors from intrapulmonary metastasis for lung cancer diagnosis. *J Cancer Res Clin Oncol*. 2020;146:2359–2367. https://doi.org/10.1007/s00432-020-03227-5
34. Duan J, Ge M, Peng J, et al. Application of large-scale targeted sequencing to distinguish multiple lung primary tumors from intrapulmonary metastases. *Sci Rep*. 2020;10:18840. https://doi.org/10.1038/s41598-020-75935-4
35. Dacic S, Cao X, Bota-Rabassedas N, et al. Genomic staging of multifocal lung squamous cell carcinomas is independent of the comprehensive morphologic assessment. *J Thorac Oncol*. 2024;19:273–284. https://doi.org/10.1016/j.jtho.2023.09.275
36. Jamal-Hanjani M, Wilson GA, McGranahan N, et al. Tracking the evolution of non-small-cell lung cancer. *N Engl J Med*. 2017;376:2109–2121. https://doi.org/10.1056/NEJMoa1616288

37. Schrader KA, Cheng DT, Joseph V, et al. Germline variants in targeted tumor sequencing using matched normal DNA. *JAMA Oncol*. 2016;2:104–111. https://doi.org/10.1001/jamaoncol.2015.5208
38. Ptashkin RN, Mandelker DL, Coombs CC, et al. Prevalence of clonal hematopoiesis mutations in tumor-only clinical genomic profiling of solid tumors. *JAMA Oncol*. 2018;4:1589–1593. https://doi.org/10.1001/jamaoncol.2018.2297
39. Karczewski KJ, Francioli LC, Tiao G, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature*. 2020;581:434–443. https://doi.org/10.1038/s41586-020-2308-7
40. Sherry ST, Ward M, Sirotkin K. dbSNP-database for single nucleotide polymorphisms and other classes of minor genetic variation. *Genome Res*. 1999;9:677–679. PMID:10447503.
41. Aokage K, Ishii G, Yoshida J, et al. Histological progression of small intrapulmonary metastatic tumor from primary lung adenocarcinoma. *Pathol Int*. 2010;60:765–773. https://doi.org/10.1111/j.1440-1827.2010.02596.x
42. Lui NS, Benson J, He H, et al. Sub-solid lung adenocarcinoma in Asian versus Caucasian patients: different biology but similar outcomes. *J Thorac Dis*. 2020;12:2161–2171. https://doi.org/10.21037/jtd.2020.04.37
43. Karasaki T, Moore DA, Veeriah S, et al. Evolutionary characterization of lung adenocarcinoma morphology in TRACERx. *Nat Med*. 2023;29:833–845. https://doi.org/10.1038/s41591-023-02230-w
44. McGranahan N, Swanton C. Clonal heterogeneity and tumor evolution: past, present, and the future. *Cell*. 2017;168:613–628. https://doi.org/10.1016/j.cell.2017.01.018
45. Oxnard GR, Miller VA, Robson ME, et al. Screening for germline EGFR T790M mutations through lung cancer genotyping. *J Thorac Oncol*. 2012;7:1049–1052. https://doi.org/10.1097/JTO.0b013e318250ed9d
46. Oxnard GR, Chen R, Pharr JC, et al. Germline EGFR mutations and familial lung cancer. *J Clin Oncol*. 2023;41:5274–5284. https://doi.org/10.1200/JCO.23.01372
47. Goodwin D, Rathi V, Conron M, Wright GM. Genomic and clinical significance of multiple primary lung cancers as determined by next-generation sequencing. *J Thorac Oncol*. 2021;16:1166–1175. https://doi.org/10.1016/j.jtho.2021.03.018
48. Donfrancesco E, Yvorel V, Casteillo F, et al. Histopathological and molecular study for synchronous lung adenocarcinoma staging. *Virchows Arch*. 2020;476:835–842. https://doi.org/10.1007/s00428-019-02736-0
49. Araujo-Filho JAB, Chang J, Mayoral M, et al. Are there imaging characteristics that can distinguish separate primary lung carcinomas from intrapulmonary metastases using next-generation sequencing as a gold standard? *Lung Cancer*. 2021;153:158–164. https://doi.org/10.1016/j.lungcan.2021.01.019
50. Yang SR, Chang JC, Leduc C, et al. Invasive mucinous adenocarcinomas with spatially separate lung lesions: analysis of clonal relationship by comparative molecular profiling. *J Thorac Oncol*. 2021;16:1188–1199. https://doi.org/10.1016/j.jtho.2021.03.023
51. Kim M, Hwang J, Kim KA, et al. Genomic characteristics of invasive mucinous adenocarcinoma of the lung with multiple pulmonary sites of involvement. *Mod Pathol*. 2022;35:202–209. https://doi.org/10.1038/s41379-021-00872-0
52. Buettner R. Invasive mucinous adenocarcinoma: genetic insights into a lung cancer entity with distinct clinical behavior and genomic features. *Mod Pathol*. 2022;35:138–139. https://doi.org/10.1038/s41379-021-00945-0
53. Li R, Li X, Xue R, et al. Early metastasis detected in patients with multifocal pulmonary ground-glass opacities (GGOs). *Thorax*. 2018;73:290–292. https://doi.org/10.1136/thoraxjnl-2017-210169
54. Liu M, He WX, Song N, Yang Y, Zhang P, Jiang GN. Discrepancy of epidermal growth factor receptor mutation in lung adenocarcinoma presenting as multiple ground-glass opacities. *Eur J Cardiothorac Surg*. 2016;50:909–913. https://doi.org/10.1093/ejcts/ezw113
55. Liu Y, Zhang J, Li L, et al. Genomic heterogeneity of multiple synchronous lung cancer. *Nat Commun*. 2016;7:13200. https://doi.org/10.1038/ncomms13200