Translational Oncology
www.transonc.com

Volume 10 Number xx Month 2017 pp. 442–449 442

Utility of Genomic Analysis in Differentiating Synchronous and Metachronous Lung Adenocarcinomas from Primary Adenocarcinomas with Intrapulmonary Metastasis[1,2]

Jad Saab, Hamid Zia, Susan Mathew, Michael Kluk, Navneet Narula and Helen Fernandes

Department of Pathology and Laboratory Medicine, New York-Presbyterian Hospital-Weill Cornell Medicine, New York, NY 10065

Abstract

Distinguishing synchronous and metachronous primary lung adenocarcinomas from adenocarcinomas with intrapulmonary metastasis is essential for optimal patient management. In this study, multiple lung adenocarcinomas occurring in the same patient were evaluated using comprehensive histopathologic evaluation supplemented with molecular analysis. The cohort included 18 patients with a total of 52 lung adenocarcinomas. Eleven patients had a new diagnosis of multiple adenocarcinomas in the same lobe ($n = 5$) or different lobe ($n = 6$). Seven patients had a history of lung cancer and developed multiple new tumors. The final diagnosis was made in resection specimens ($n = 49$), fine needle aspiration ($n = 2$), and biopsy ($n = 1$). Adenocarcinomas were non-mucinous, and histopathologic comparison of tumors was performed. All tumors save for one were subjected to *ALK* gene rearrangement testing and targeted Next Generation Sequencing (NGS). Using clinical, radiologic, and morphologic features, a confident conclusion favoring synchronous/metachronous or metastatic disease was made in 65% of patients. Cases that proved challenging included ones with more than three tumors showing overlapping growth patterns and lacking a predominant lepidic component. Genomic signatures unique to each tumor were helpful in determining the relationship of multiple carcinomas in 72% of patients. Collectively, morphologic and genomic data proved to be of greater value and achieved a conclusive diagnosis in 94% of patients. Assessment of the genomic profiles of multiple lung adenocarcinomas complements the histological findings, enabling a more comprehensive assessment of synchronous, metachronous, and metastatic lesions in most patients, thereby improving staging accuracy. Targeted NGS can identify genetic alterations with therapeutic implications.

Translational Oncology (2017) 10, 442–449

Introduction

Lung cancer remains the leading cause of cancer-related death in both men and women in the United States with an estimated 221,000 new diagnoses and 158,000 deaths in 2015 [1]. Of all the subtypes of lung carcinoma, adenocarcinoma has shown a dramatic rise in incidence worldwide and currently accounts for approximately half of all newly diagnosed primary lung malignancies [2–4]. Although most patients are diagnosed with a single primary lung adenocarcinoma, the frequency of identifying two or more adenocarcinomas at presentation is not uncommon and has an estimated incidence ranging from 1% to 8% [5–13]. Depending on the stage at

Address all correspondence to: Helen Fernandes, PhD, Department of Pathology and Cell Biology, Laboratory of Personalized Genomic Medicine, Columbia University Medical Center, New York, NY 10032.
E-mail: hf2340@cumc.columbia.edu

[1] Conflict of Interest and Disclosure: The authors have no disclosures.

[2] Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.
Received 15 December 2016; Revised 15 February 2017; Accepted 23 February 2017

http://dx.doi.org/10.1016/j.tranon.2017.02.009

presentation, 20% to 52% of lung cancer patients eventually develop recurrent locoregional or metastatic disease [14,15]. Moreover, the incidence of developing a second primary lung cancer ranges from 1% to 4% per patient-year [8,16–19].

The diagnostic workup and treatment approach for a solitary primary lung adenocarcinoma are well established; however, identification of multiple carcinomas at presentation introduces new challenges both from a clinicopathologic standpoint and in relation to treatment strategy [20]. In the absence of a history of lung cancer, multiple adenocarcinomas could arise independently or may represent an advanced stage at presentation. In the setting of a previously diagnosed lung adenocarcinoma, newly identified lung cancers may represent new independent primary cancers, a new independent primary carcinoma with intrapulmonary metastasis, or recurrence of the previously diagnosed tumor.

In 1975, Martini and Melamed developed criteria defining two categories of multiple primary lung carcinomas: "synchronous" and "metachronous" [7]. Briefly, synchronous and metachronous refer to independent, unrelated, primary lung carcinomas arising simultaneously or at different points in time, respectively. The definition holds even when the histology is comparable if the tumors occur in different segments in the absence of common lymphatic involvement, and mediastinal or systemic metastases [7,20,21]. In patients with a history of lung cancer, a newly diagnosed lung carcinoma is considered metachronous if it is histologically different from the prior tumor. Identical histology does not preclude this categorization as long as the new carcinoma involves a different lobe, lacks common lymphatic involvement and extrapulmonary metastases, is detected 2 or more years later, or is associated with an *in situ* component [7,20,21]. The concept of comparable histology, however, can be challenging due to interobserver variability. The classification strategy set forth by the International Association for the Study of Lung Cancer (IASLC) recommended recording the percentages of different histologic patterns in lung adenocarcinoma. This allowed for more precise histologic comparison between multiple tumors and a greater level of consistency among pathologists [22,23]. The effort to distinguish multiple lung carcinomas was further reinforced by studies utilizing immunohistochemistry [24], DNA microsatellite analysis [25,26], clonality analysis [27], comparative genomic hybridization [28], and targeted gene sequencing [29–33].

The aim of this study is to determine the utility of targeted gene profiling using Next Generation Sequencing (NGS) in aiding the histomorphologic assessment for the classification of multiple lung adenocarcinomas as synchronous, metachronous, or metastatic.

Materials and Methods

Study Group

Pulmonary adenocarcinomas histologically diagnosed and subject to NGS between January and December 2015 were identified by computerized search of existing records. During this period, all specimens including cytology, biopsy, or resection were subject to NGS analysis. Of the 380 patients with lung tumors diagnosed as nonmucinous lung adenocarcinomas and in which targeted genomic profiling was successful, 18 with two or more concurrent lung tumors were identified for analysis. Clinical, demographic, and radiographic information including the number and location of tumors, history of lung carcinoma, and history of systemic therapy was obtained. Tumor size determination and measurement of distance of the tumors from one another were performed at the time of gross assessment and microscopic examination and were correlated with the radiologic findings. In patients with a history of lung adenocarcinoma, the original slides were reviewed when available, and NGS was performed on the paraffin-embedded tumor tissue sections. Approval for the study was obtained from the Institutional Review Board of from the New York Presbyterian Hospital-Weill Cornell Medicine in New York, NY.

Histologic Features and Relatedness

Tumor-containing hematoxylin and eosin–stained slides from formalin-fixed, paraffin-embedded tissue were blindly reviewed by two pathologists. The number of slides reviewed per case ranged from 2 to 10. Histologic subtyping was performed in all resection specimens showing a heterogeneous mixture of morphologic patterns as recommended by the IASLC [22]. Histologic patterns were quantified in 5% increments and included acinar, papillary, micropapillary, solid, cribriform, and lepidic. Resected adenocarcinomas with a purely lepidic growth pattern, an acinar or papillary component, and a micropapillary or solid component were graded as well, intermediate, and poorly differentiated, respectively [22,34,35]. The clinical, radiologic, and morphological features of each patient's tumors were used to categorize them as synchronous, metachronous, and metastatic. Immunohistochemical stains including TTF-1 (thyroid transcription factor-1, monoclonal: 8G7G3 Thermo Fisher Scientific), Napsin-A (polyclonal Cell Marque), and p40 (polyclonal Biocare Medical) were used to differentiate glandular from squamous differentiation in a subset of cases.

The relatedness of multiple lung carcinomas was determined using the following algorithm. Invasive adenocarcinomas exhibiting similar morphologies, primary growth patterns, and cytologic features; lacking a conspicuous lepidic component; and demonstrating identical NGS results were considered metastatic in nature irrespective of their location. Tumors with different predominant growth patterns and cytology arising in different lung lobes and exhibiting different NGS findings were considered independent and synchronous if identified at the same time and metachronous if one tumor was diagnosed at a different point in time. Tumors with similar growth patterns but distinct genomic findings were favored to be independent of one another [28,36]. Lastly, in the setting of identical molecular findings, tumors were favored to be independent if they exhibited different growth patterns, predominant lepidic growth, and/or dissimilar cytology.

Genomic and Cytogenetic Analysis

Ten unstained slides with 5-μm sections were obtained from formalin-fixed, paraffin-embedded tumor tissue. The tumor-enriched area was encircled on the hematoxylin and eosin–stained slides, and the neoplastic cellularity of the selected area was estimated by a pathologist. The scored slide was used to guide the macrodissection of tumor cells from the unstained slides. DNA was extracted using the Maxwell 16 FFPE DNA kit (Promega Corp., WI). NGS using 1 to 10 ng of input DNA was performed on the Ion Torrent Personal Genome Machine using the AmpliSeq Cancer Hotspot Panel v2 (Thermo Fisher Scientific, MA). Sequencing data were analyzed using the Variant Caller v4.4 (Thermo Fisher Scientific, MA). The NGS assay used for the study was validated and approved for accuracy, reproducibility, precision, and sensitivity in accordance with the guidelines of the New York State department of health. For cytogenetic

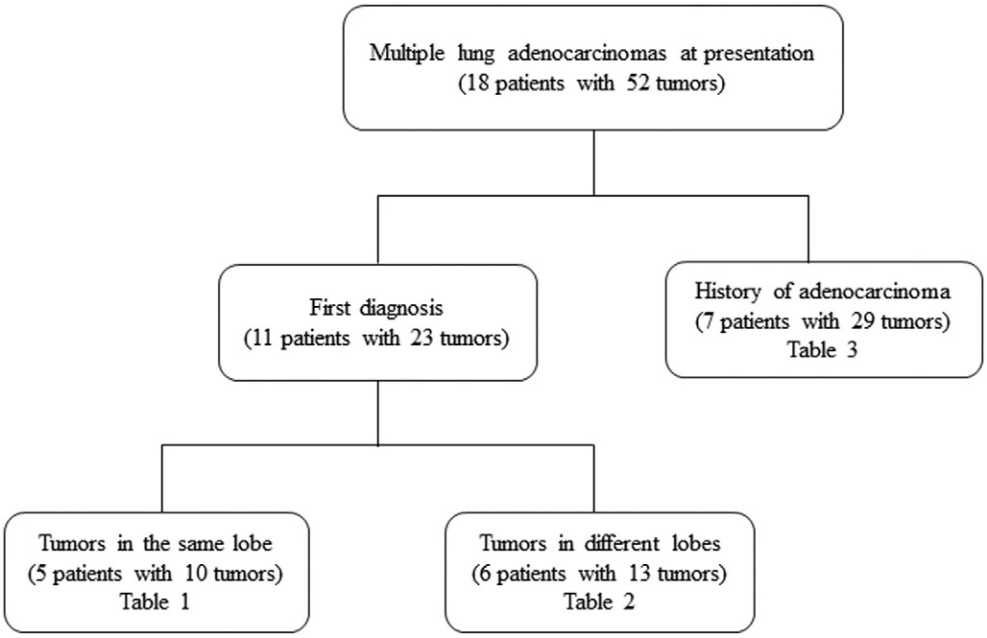

Figure 1. Approach used in studying patients presenting with multiple lung adenocarcinomas. Patients with a new diagnosis were divided into two categories: those whose tumors were present in the same lung lobe (Table 1) and those with tumors in different lobes (Table 2). Patients with a history of lung adenocarcinoma who were diagnosed with new multiple adenocarcinomas on follow-up were studied separately (Table 3).

analysis, the LSI *ALK* dual-color break-apart rearrangement probe (Vysis/Abbott Molecular Inc., Des Plaines, IL) was used to test for rearrangements of the *ALK* gene. NGS and *ALK* FISH testing were performed in all tumors except one due to the unavailability of the tissue block.

Results

Eighteen patients comprising 13 women and 5 men with a total of 52 lung adenocarcinomas were included in the study. Presenting symptoms included cough (n = 7) and shortness of breath (n = 2); identification of the tumors in the remaining patients either was incidental (n = 5) or was detected on annual chest radiography (n = 4). The mean age at initial diagnosis was 66 years (median: 67, range: 51-78). A history of former or current smoking was present in 15/18 (83%) patients. Seventeen of the 18 patients harbored clinically relevant variants in one or more of the tumors. Patients were divided into three categories depending on tumor location and history of prior lung carcinoma (Figure 1). Eleven of the 18 patients were diagnosed with multiple lung adenocarcinomas at initial presentation. A clinical decision was made to treat the tumors as separate primaries in all patients. Five of the 11 patients presented with two tumors in the same lung lobe (Table 1). Different growth patterns were noted in the tumors of three patients. In the remaining two patients, the growth patterns were similar; however, cytoplasmic and/or nuclear features were dissimilar in the paired tumor samples (Figure 2). Molecular alterations were dissimilar in the tumors of the four patients that harbored variants (Table 1). Based on the combined histologic and molecular findings, all tumors were considered as synchronous lung adenocarcinomas.

Of the 11 patients presenting with multiple lung adenocarcinomas at initial presentation, 6 presented with tumors in different lung lobes (Table 2). Histologic comparison was possible in four patients and revealed distinct growth patterns and dissimilar cytoplasmic and/or

Table 1. Clinical, Pathologic, and Molecular Characteristic of Patients Newly Diagnosed with Multiple Lung Adenocarcinomas in the Same Lobe

Age/Sex	Site	Procedure	Size (cm)	DX	Growth Patterns*	Cytology/Stroma	NGS Results	Interpretation
70/F	RU	Segment	1.2	AC	Acinar, lepidic, solid	Different	*EGFR* G719C, *EGFR* S768I, *TP53* A159P	Synchronous
		Segment	0.6	AIS	Lepidic		*KRAS* Q61L	
69/M	RU	Lobe	1.5	AC	Lepidic, acinar	Different	*KRAS* Q61H	Synchronous
			0.4	MIA	Lepidic, acinar		NONE	
51/M	RU	Lobe	4	AC	Solid, lepidic, micropapillary	Different	*TP53* G266*	Synchronous
			1.2	AC	Solid, lepidic, micropapillary		*BRAF* V600E	
68/M	LU	Wedge	1.8	AC	Acinar, lepidic	Different	NONE	Synchronous
		Wedge	0.6	AC	Lepidic, acinar, micropapillary		NONE	
59/F	RL	Lobe	2.4	AC	Acinar, micropapillary, lepidic	Different	*KRAS* G12D, *BRAF* V600E	Synchronous
			2	AC	Papillary, acinar, lepidic		*KRAS* G12 V	

Legend: *AC*, adenocarcinoma; *AIS*, adenocarcinoma in situ; *DX*, diagnosis; *F*, female; *FNA*, fine needle aspiration; *LU*, left upper lobe; *M*, male; *MIA*, minimally invasive adenocarcinoma; *RL*, right lower lobe; *RU*, right upper lobe.

* The proportion of different growth patterns was categorized from most common (predominant pattern) to least common.

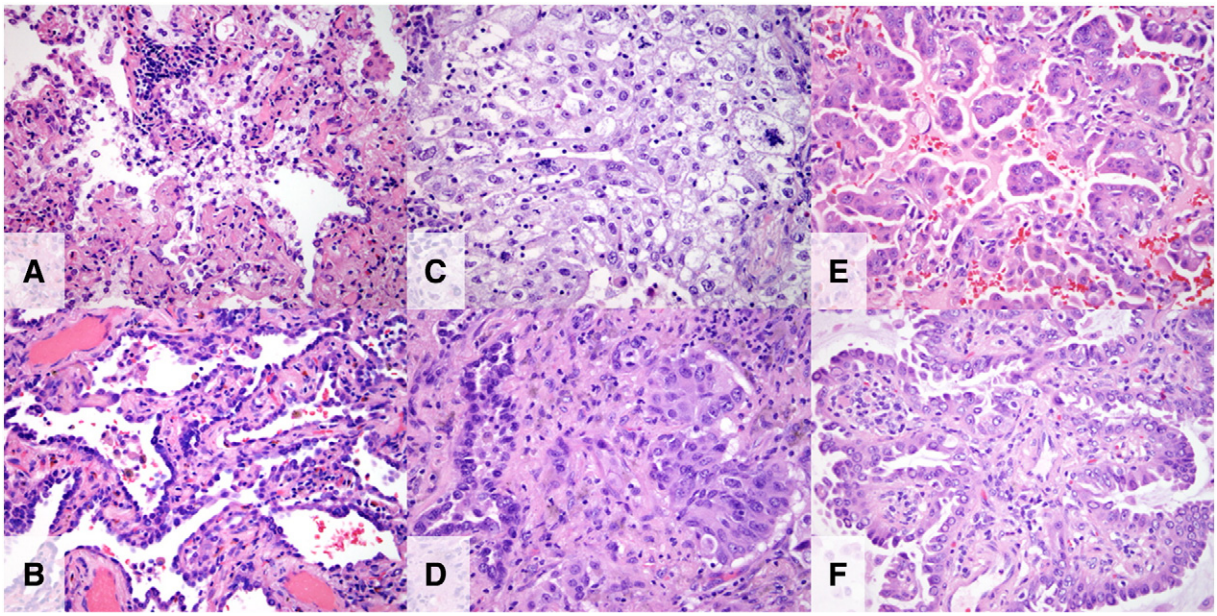

Figure 2. Paired tumors from three patients arising in the same lobe and showing distinct cytological and molecular features (A-B: Table 1, patient #2, C-D: Table 1, patient #3, E-F: Table 1, patient #5). Although both tumors showed combined acinar and lepidic growth, one tumor featured clear, vacuolated cytoplasm (A, 400×), whereas the other showed scant eosinophilic cytoplasm (B, 400×). This patient's tumors both showed a mixture of solid, micropapillary, and lepidic growth; however, one featured pleomorphic cells with granular and vacuolated cytoplasm (C, 400×), whereas the other had more uniform tumor cells with dense eosinophilic cytoplasm (D, 400×). In this patient, one tumor was acinar predominant with focal micropapillary areas (E, 400×), whereas the other was papillary predominant (F, 400×).

nuclear features (Figure 3). Despite the presence of lymphatic involvement and regional nodal disease in three of these patients, distinct molecular alterations were identified. Based on these findings, these four patients were considered to have synchronous tumors. One patient was a poor surgical candidate and was diagnosed with two adenocarcinomas on FNA and biopsy; histologic comparison was not possible, but molecular findings were different, supporting categorization as synchronous. The last patient in this group presented with three tumors involving different lobes; two tumors were resected, and the third tumor was diagnosed by FNA and treated with radiation and chemotherapy. The resected tumors exhibited similar growth patterns but distinct cytologic features and molecular alterations (*KRAS* G12C and an *EGFR* exon 19 deletion). The tumor with the *KRAS* gene variant showed lymphatic involvement and nodal disease. The nonresected tumor also harbored a *KRAS* G12C variant, possibly representing an intrapulmonary metastasis.

The clinicopathologic characteristics and molecular signatures of the seven remaining patients with a prior history of lung adenocarcinoma and new multiple lung adenocarcinomas are

Table 2. Clinical, Pathologic, and Molecular Characteristic of Patients Newly Diagnosed with Multiple Lung Adenocarcinomas in Different Lobes

Age/Sex	Site	Procedure	Size (cm)	DX	Growth Patterns*	Cytology/Stroma	NGS Results	Interpretation
71/F	LU	Lobe	3	AC	Solid, acinar, micropapillary	Different	*EGFR* L747_T751del15	Synchronous
	LL	Segment	1.5	AC	Acinar, lepidic		*EGFR* L858R	
64/M	LL	Lobe	4.5	AC†	Solid, micropapillary	Different	*KRAS* G12 V	Synchronous
	RL	Lobe	2.5	AC	Acinar, lepidic		*KRAS* Q61H, *TP53* G245 V	
66/F	LL	Lobe	2.5	AC	Acinar, lepidic, papillary	Different	*PIK3CA* H1047L	Synchronous
	RL	Wedge	1.5	AC‡	Acinar, micropapillary, lepidic		*EGFR* E746_A750del15	
54/M	LU	Wedge	1.1	AC	Acinar, lepidic	Different	*EGFR* L858R	Synchronous
	RU	Lobe	3.2	AC†	Solid, acinar		*EGFR* E746_A750del15	
64/F	RM	Biopsy	<1	AC	N/A	N/A	*KRAS* G12C	Synchronous
	LL	FNA	2.5	AC	N/A	N/A	*TP53* R273C	
66/F	LU	Wedge	2.9	AC†	Acinar, lepidic, micropapillary	Different	*KRAS* G12C	Synchronous
	LL	Segment	1.6	AC	Acinar, lepidic, micropapillary		*EGFR* L747_T751del15, *PIK3CA* T1025A	
	RU	FNA	1	AC	N/A	N/A	*KRAS* G12C	Undetermined

Legend: *LL*, left lower lobe; *N/A*, not applicable; *RM*, right middle lobe.

* The proportion of different growth patterns was categorized from most common (predominant pattern) to least common.

† N1 disease identified.

‡ N2 disease identified.

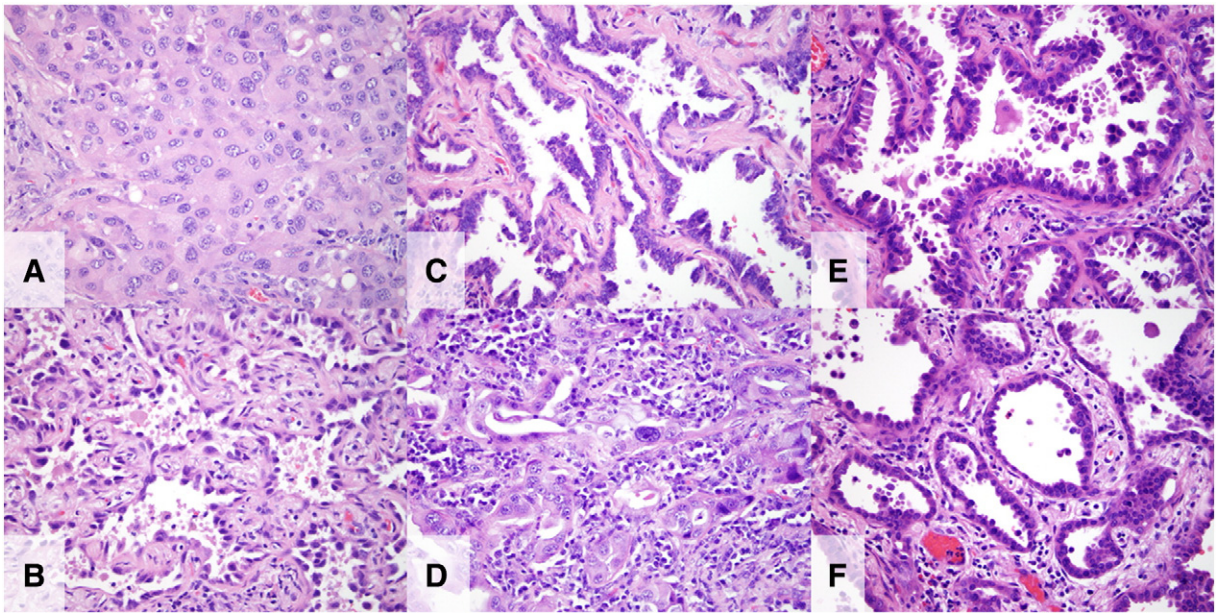

Figure 3. Paired tumors from three patients arising in different lobes and showing distinct cytological and molecular features (A-B: Table 2, patient #1, C-D: Table 2, patient #3, E-F: Table 2, patient #6). One tumor shows a predominantly solid growth pattern with moderately atypical nuclei and abundant eosinophilic cytoplasm (A, 400×), whereas the second tumor exhibits an acinar growth pattern with hyperchromatic hobnailed nuclei (B, 400×). Although both tumors exhibit an acinar growth pattern, one has low-grade nuclei and a pauci-inflammatory stroma (C, 400×), whereas the other has pleomorphic nuclei and an inflamed stroma (D, 400×). Two tumors both featuring acinar and micropapillary growth; however, one tumor has columnar cells with abundant cytoplasm, apical snouts, and hobnailed nuclei (E, 400×), features not readily identified in the other tumor (F, 400×).

highlighted in Table 3. All patients had node-negative disease on initial presentation. Patients subsequently presented with two to five new tumors including adenocarcinoma (n = 16), adenocarcinoma *in situ* (n = 3), and minimally invasive adenocarcinoma (n = 1). The tumors involved the contralateral lung in six patients and another lobe of the same lung in one patient. The time spanning the initial and the new diagnosis ranged from 1 to 10 years. Molecular testing was performed on all but one specimen, and histologic data were available

Table 3. Characteristics of Patients with a History of Lung Adenocarcinoma and New Multiple Lung Adenocarcinomas

Age/Sex	Site	Procedure	Size (cm)	DX	Growth Patterns *	Cytology/Stroma	NGS Results	Interpretation
79/F	LL	Wedge	1	AC	Acinar, lepidic, solid, micropapillary	Similar	*EGFR* L747_T751del15	Intrapulmonary metastasis
	LL	Wedge	1.5	AC	Micropapillary, cribriform, acinar, solid		*EGFR* L747_T751del15	
71/F	LU	Lobe	2.7	AC	Acinar, lepidic	Different	*EGFR* L747_T751del15	Tumor at first presentation
		Wedge	0.5	MIA	Lepidic, acinar		*EGFR* L858R	
76/F	RU	Wedge	1.9	AC	Lepidic, acinar	Similar	*EGFR* L858R	Metachronous
		Wedge	0.9	AIS	Lepidic		*MET* T1010I	
72/F	LU	Lobe	2.2	AC	Lepidic, acinar	Different	*TP53* N131I	Tumor at first presentation
	RU	Wedge	1.5	AC	Acinar, solid, lepidic		*TP53* A159V	
81/F	RL	Wedge	2.7	AIS	Lepidic	Different	*KRAS* G12C	Metachronous
		Wedge	2.5	AIS	Lepidic		*KRAS* G12C	
71/F	LU	Lobe	2.8	AC	Solid		Tissue not available	Tumor at first presentation
69/F	LL	Wedge	1.2	AC	Solid, acinar		*TP53* R273L	Metachronous
	LU	Wedge	0.9	AC	Lepidic, acinar	Different	*KRAS* G12C	
67/F	RU	Wedge	0.6	AC	Acinar, lepidic		*KRAS* G12A, *CTNNB1* S33F	Tumors at first presentation
	RL	Lobe	2.2	AC	Papillary, lepidic		*KRAS* G12C	
68/F	RU	Segment	1.1	AC	Lepidic, acinar		*KRAS* G12C	Metachronous
	RU	Segment	1.9	AC	Acinar, lepidic		*BRAF* D594G, *KIT* M541 L	
67/F	RL	Segment	0.8	AC	Cribriform, solid	Different	*TP53* S215I	Tumors at first presentation
	LU	Wedge	0.9	AC	Acinar, micropapillary		*KIT* M541 L	
	LU	Wedge	1.9	AC	Lepidic, papillary		*KRAS* G12C, *TP53* V157F, *KIT* M541 L	
	RL	Wedge	0.9	AC	Micropapillary, acinar		*KRAS* G12D	Metachronous
78/F	RL	Segment	1	AC	Acinar	Different	*KRAS* G12 V, *TP53* R280T	Metachronous
	RL	Segment	1	AC	Acinar, lepidic, cribriform		*KRAS* G12C, *TP53* R280T	Favor metachronous
	RL	Segment	0.7	AC	Cribriform, lepidic		*KRAS* G12C, *TP53* R280T	
	RU	Wedge	0.5	AC	Acinar, micropapillary		*NRAS* Q61R, *TP53* G334 V	Metachronous
	LU	Lobe	2.8	AC	Cribriform, micropapillary, lepidic		*KRAS* G12A, *STK11* F354 L	Tumor at first presentation
72/F	LU	Wedge	1.5	AC	Lepidic, acinar, papillary		*KRAS* G13D, *GNAS* Q227L	Metachronous
	LU	Wedge	2	AC	Acinar, lepidic, papillary	Similar	*KRAS* G12C	
62/F	RU	Lobe	2	AC	Lepidic, papillary		*EGFR* L861Q	Tumor at first presentation

* The proportion of different growth patterns was categorized from most common (predominant pattern) to least common.

in all patients. Of the seven patients, six were determined to have metachronous tumors; findings favoring classification as metachronous included *in situ* adenocarcinoma, dissimilar predominant growth patterns, distinct cytologic features, and distinct molecular findings (Figure 4). The newly diagnosed tumor pair in one patient showed an identical *EGFR* exon 19 deletion and similar growth patterns and was favored to represent an intrapulmonary metastasis. The same deletion was identified in the patient's original tumor diagnosed 8 years earlier; however, the tumor showed dissimilar cytologic features, and a definitive interpretation regarding the relatedness of all three tumors could not be made (Table 3, patient #1). None of the tumors tested harbored *ALK* gene rearrangements.

In summary, all tumors arising in the same lung lobe and identified at the same time in patients without a history of lung cancer were interpreted as independent primary malignancies (Table 1). Similarly, in patients without a history of lung cancer, all simultaneously detected tumors arising in different lung lobes were also determined to be independent primary adenocarcinomas when morphologic assessment was possible (Table 2). Newly diagnosed carcinomas in patients with a history of lung adenocarcinoma were unrelated to the original tumor in all but one patient (Table 3).

Discussion

Identification of two or more primary lung carcinomas at presentation is not uncommon, with an incidence ranging from 5.7% to 11.5%. Specifically, a diagnosis of multiple primary lung adenocarcinomas can be seen in up to 8% of patients [12,17,37,38]. Determining whether these tumors are truly independent can be challenging given that lung adenocarcinomas frequently displays a spectrum of histologic subtypes, even within the same tumor, and tumors may show histologic overlap. Documented criteria were originally proposed to address this matter but may lack the power to differentiate between a metastatic lesion and a second primary lung cancer, a distinction that directly impacts prognosis and treatment options [6,20,22,27–29,36]. In 2011, the IASLC aimed to unify the terminology and diagnostic criteria for lung adenocarcinoma and recommended recording the percentages of the different histologic patterns and noting certain cytologic and stromal features [22,23,34,35,39–46]. This diagnostic approach allowed for a more accurate histologic comparison between multiple adenocarcinomas and was potentially useful in distinguishing multiple primary tumors from intrapulmonary metastases in two thirds of the cases in our study. Nevertheless, histologic overlap in predominant and secondary growth patterns was encountered in the tumors of three patients (Table 1, patient #3; Table 2, patient #6; Table 3, patient #6).

Cytologic and stromal features frequently differed in tumors with overlapping growth patterns. Conclusions regarding the relatedness of multiple lung adenocarcinomas that were made by assessing growth patterns agreed with those reached by examining cytologic/stromal features in 9 of 17 cases (53%). Using clinical, radiologic, and morphologic features, a confident conclusion favoring synchronous/metachronous or metastatic disease was made in 11 of 17 evaluable cases (65%). Cases that proved challenging included patients with more than three tumors showing overlapping growth patterns and lacking a predominant lepidic component. Genomic data were able to ascertain the relationship of carcinomas in 13 of 18 patients (72%). When used together, clinicopathologic and molecular data proved to be of greater value in this endeavor, and a conclusion was reached in 17 of 18 patients (94%). Determining the relationship of multiple tumors was challenging in patient #6 in Table 2, where one tumor was diagnosed by FNA alone and featured the same genomic variant as another tumor.

In addition to its potential therapeutic implications, genomic testing using capture-based and amplicon-based technologies has been used by several studies attempting to unravel the relatedness of multiple lung carcinomas. Assessment of *TP53* and *EGFR* mutations by clonality analysis revealed that approximately half of all cases display different clonality [27]. Mutational profiling evaluating *EGFR*, *KRAS*, and *TP53* was shown to improve discrimination between independent multiple primary lung carcinomas and intrapulmonary metastasis [29–33]. These findings along with studies utilizing DNA microsatellite analysis [25,26], loss of heterozygosity [47], comparative genomic hybridization [28], and immunohistochemistry [24] highlight the importance of using genomic profiling in conjunction with clinicopathological criteria in order to better differentiate independent primary tumors from intrapulmonary metastasis [25–29,36,48,49]. Recent investigations stress the significance of a multidisciplinary approach for studying the clonal relationship of multifocal lung tumors [50].

NGS assays for detection of genomic alterations with therapeutic, diagnostic, and prognostic significance in lung cancer are now routinely utilized in major medical centers. Targeted NGS panels are robust and sensitive for identification of somatic variants in cancer hotspots or the entire coding region of a gene. Utilizing a 50-gene

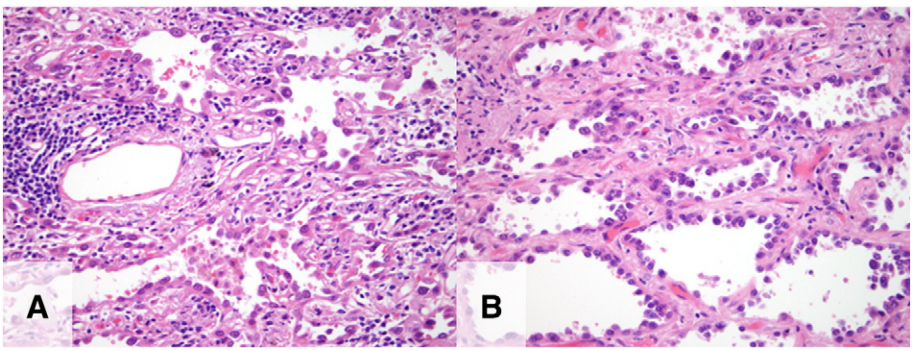

Figure 4. A 72-year-old woman with a history of lung adenocarcinoma presenting with two left upper lobe tumors having similar growth patterns and cytologic features (Table 3, patient #7). One tumor showed *KRAS* G13D and *GNAS* Q227L mutations (A, 400×), whereas the second tumor had a *KRAS* G12C mutation (B, 400×). The tumors were classified as independent of one another and unrelated to the prior tumor.

targeted NGS panel, our pilot study showed that interrogation of clinically relevant variants in conjunction with histomorphologic features can be informative for assessment of synchronous, metachronous, and metastatic lesions in patients with pulmonary adenocarcinomas. We detected a total of 63 clinically relevant variants predictive of response to approved therapies and/or clinical trials, including seven deletions in the exon 19 of the *EGFR* gene. The patterns of variants from *KRAS* (n = 23), *EGFR* (n = 14), *TP53* (n = 13), *BRAF* (n = 3), *KIT* (n = 3), and *PIK3CA* (n = 2) genes and single variants of *NRAS*, *STK11*, *MET, CTNNB1*, and *GNAS* genes were comparable to genomic signatures of the 700 lung adenocarcinomas that have been sequenced in our clinical molecular pathology laboratory to date. Interestingly, of the 23 *KRAS* variants detected, 19 were the more deleterious transversion mutations, and all occurred in current or former smokers as previously described [51]. Studies on larger patient cohorts with long-term follow-up are needed to determine whether *KRAS* transversion mutations are more common in the setting of synchronous lung adenocarcinomas and if they portend more aggressive disease.

Several observations can be made about the utility of NGS in classifying multiple lung adenocarcinomas. Collectively, our observations suggest that most multiple lung adenocarcinomas arising in patients without a history of lung cancer are truly independent primaries whether they arise in the same or different lung lobe, even in the setting of regional nodal disease. These tumors are therefore best regarded as separate primaries and should be staged and treated as such. Similarly, the majority of tumors detected in patients with a history of lung adenocarcinoma are distinct from the original primary and from each other. When histologic comparison is not possible, such as in patients unable to tolerate resection, NGS may provide prognostic and predictive value. A limitation of the current study is the relatively small number of targets in the gene panel and its inability to interrogate fusion and copy number alterations, both of which have the capability to further discriminate primary lung adenocarcinomas from metastases [52]. Studies with a larger cohort of patients and more informative panels, long-term follow-up, and survival data would be helpful in substantiating our observations and those of other investigators.

Conclusions

Our findings highlight the importance of using clinicopathological criteria in conjunction with genomic profiling for better elucidation of the differences between independent primary tumors from intrapulmonary metastasis and for identifying molecular alterations with therapeutic implications. NGS panels provide surrogate genomic information that is important for classification of patients with multiple lung adenocarcinomas with similar morphology and are less prone to the potential interobserver variability associated with assessment of tumor growth patterns and cytology.

References

[1] Siegel RL, Miller KD, and Jemal A (2015). Cancer statistics, 2015. *CA Cancer J Clin* **65**, 5–29.

[2] Travis WD (2011). Pathology of lung cancer. *Clin Chest Med* **32**, 669–692.

[3] Travis WD (2002). Pathology of lung cancer. *Clin Chest Med* **23**, 65–81.

[4] Houston KA, Henley SJ, Li J, White MC, and Richards TB (2014). Patterns in lung cancer incidence rates and trends by histologic type in the United States, 2004-2009. *Lung Cancer* **86**, 22–28.

[5] Kim SW, Kong KA, Kim DY, Ryu YJ, Lee JH, and Chang JH (2015). Multiple primary cancers involving lung cancer at a single tertiary hospital: clinical features and prognosis. *Thorac Cancer* **6**, 159–165.

[6] Chang YL, Wu CT, and Lee YC (2007). Surgical treatment of synchronous multiple primary lung cancers: experience of 92 patients. *J Thorac Cardiovasc Surg* **34**, 630–637.

[7] Martini N and Melamed MR (1975). Multiple primary lung cancers. *J Thorac Cardiovasc Surg* **70**, 606–612.

[8] Deschamps C, Pairolero PC, Trastek VF, and Payne WS (1990). Multiple primary lung cancers. Results of surgical treatment. *J Thorac Cardiovasc Surg* **99**, 769–777.

[9] Carey FA, Donnelly SC, Walker WS, Cameron EW, and Lamb D (1993). Synchronous primary lung cancers: prevalence in surgical material and clinical implications. *Thorax* **48**, 344–346.

[10] Pommier RF, Vetto JT, Lee JT, and Johnston KM (1996). Synchronous non–small cell lung cancers. *Am J Surg* **171**, 521–524.

[11] Ferguson MK, DeMeester TR, DesLauriers J, Little AG, Piraux M, and Golomb H (1985). Diagnosis and management of synchronous lung cancers. *J Thorac Cardiovasc Surg* **89**, 378–385.

[12] Nakata M, Sawada S, Yamashita M, Saeki H, Kurita A, Takashima S, and Tanemoto K (2004). Surgical treatments for multiple primary adenocarcinoma of the lung. *Ann Thorac Surg* **78**, 1194–1199.

[13] Zwirewich CV, Miller RR, and Müller NL (1990). Multicentric adenocarcinoma of the lung: CT-pathologic correlation. *Radiology* **176**, 185–190.

[14] Virgo KS, McKirgan LW, Caputo MC, Mahurin DM, Chao LC, Caputo NA, Naunheim KS, Flye MW, Gillespie KN, and Johnson FE (1995). Post-treatment management options for patients with lung cancer. *Ann Surg* **222**, 700–710.

[15] Lou F, Sima CS, Rusch VW, Jones DR, and Huang J (2014). Differences in patterns of recurrence in early-stage versus locally advanced non–small cell lung cancer. *Ann Thorac Surg* **98**, 1755–1760.

[16] Rice D, Kim HW, Sabichi A, Lippman S, Lee JJ, Williams B, Vaporciyan A, Smythe WR, Swisher S, and Walsh G, et al (2003). The risk of second primary tumors after resection of stage I nonsmall cell lung cancer. *Ann Thorac Surg* **76**, 1001–1007.

[17] Pairolero PC, Williams DE, Bergstralh EJ, Piehler JM, Bernatz PE, and Payne WS (1984). Postsurgical stage I bronchogenic carcinoma: morbid implications of recurrent disease. *Ann Thorac Surg* **38**, 331–338.

[18] Ribet M and Dambron P (1995). Multiple primary lung cancers. *Eur J Cardiothorac Surg* **9**, 231–236.

[19] Van Meerbeeck J, Weyler J, Thibaut A, Vansteenkiste J, Aumann J, Deneffe G, Galdermans D, Haelterman M, Joos G, and Noppen M , et al (1996). Second primary lung cancer in Flanders: frequency, clinical presentation, treatment and prognosis. *Lung Cancer* **15**, 281–295.

[20] NCCN (). Guidelines Version 1.2014 Non–Small Cell Lung Cancer. http://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf.

[21] Kozower BD, Larner JM, Detterbeck FC, and Jones DR (2013). 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* **143**, e369S–e399S.

[22] Travis WD, Brambilla E, Nicholson A, Yatabe Y, Austin JH, Beasley MB, Chirieac LR, Dacic S, Duhig E, and Flieder DB (2015). The 2015 World Health Organization classification of lung tumors impact of genetic, clinical and radiologic advances since the 2004 classification. *J Thorac Oncol* **10**, 1243–1260.

[23] Girard N, Deshpande C, Lau C, Finley D, Rusch V, Pao W, and Travis WD (2009). Comprehensive histologic assessment helps to differentiate multiple lung primary nonsmall cell carcinomas from metastases. *Am J Surg Pathol* **33**, 1752–1764.

[24] Nonami Y, Ohtuki Y, and Sasaguri S (2003). Study of the diagnostic difference between the clinical diagnostic criteria and results of immunohistochemical staining of multiple primary lung cancers. *J Cardiovasc Surg* **44**, 661–665.

[25] Dacic S, Ionescu DN, Finkelstein S, and Yousem SA (2005). Patterns of allelic loss of synchronous adenocarcinomas of the lung. *Am J Surg Pathol* **29**, 897–902.

[26] Wang X, Wang M, MacLennan GT, Abdul-Karim FW, Eble JN, Jones TD, Olobatuyi F, Eisenberg R, Cummings OW, and Zhang S, et al (2009). Evidence for common clonal origin of multifocal lung cancers. *J Natl Cancer Inst* **101**, 560–570.

[27] Chang YL, Wu CT, Lin SC, Hsiao CF, Jou YS, and Lee YC (2007). Clonality and prognostic implications of p53 and epidermal growth factor receptor somatic aberrations in multiple primary lung cancers. *Clin Cancer Res* **13**, 52–58.

[28] Girard N, Ostrovnaya I, Lau C, Park B, Ladanyi M, Finley D, Deshpande C, Rusch V, Orlow I, and Travis WD, et al (2009). Genomic and mutational profiling to assess clonal relationships between multiple nonsmall cell lung cancers. *Clin Cancer Res* **15**, 5184–5190.

[29] Girard N, Deshpande C, Azzoli CG, Rusch VW, Travis WD, Ladanyi M, and Pao W (2010). Use of epidermal growth factor receptor/Kirsten rat sarcoma 2 viral oncogene homolog mutation testing to define clonal relationships among multiple lung adenocarcinomas: comparison with clinical guidelines. *Chest* **137**, 46–52.

[30] van Rens MT, Eijken EJ, Elbers JR, Lammers JW, Tilanus MG, and Slootweg PJ (2002). p53 mutation analysis for definite diagnosis of multiple primary lung carcinoma. *Cancer* **94**, 188–196.

[31] Matsuzoe D, Hideshima T, Ohshima K, Kawahara K, Shirakusa T, and Kimura A (1999). Discrimination of double primary lung cancer from intrapulmonary metastasis by p53 gene mutation. *Br J Cancer* **79**, 1549–1552.

[32] Wang X, Christiani DC, Mark EJ, Nelson H, Wiencke JK, Gunn L, Wain JC, and Kelsey KT (1999). Carcinogen exposure p53 alteration, and K-ras mutation in synchronous multiple primary lung carcinoma. *Cancer* **85**, 1734–1739.

[33] Lau DH, Yang B, Hu R, and Benfield JR (1997). Clonal origin of multiple lung cancers: K-ras and p53 mutations determined by nonradioisotopic single-strand conformation polymorphism analysis. *Diagn Mol Pathol* **6**, 179–184.

[34] Sica G, Yoshizawa A, Sima CS, Azzoli CG, Downey RJ, Rusch VW, Travis WD, and Moreira AL (2010). A grading system of lung adenocarcinomas based on histologic pattern is predictive of disease recurrence in stage I tumors. *Am J Surg Pathol* **34**, 1155–1162.

[35] Yoshizawa A, Motoi N, Riely GJ, Sima CS, Gerald WL, Kris MG, Park BJ, Rusch VW, and Travis WD (2011). Impact of proposed IASLC/ATS/ERS classification of lung adenocarcinoma: prognostic subgroups and implications for further revision of staging based on analysis of 514 stage I cases. *Mod Pathol* **24**, 653–664.

[36] Moffatt-Bruce SD, Ross P, Leon ME, He G, Finkelstein SD, Vaida AM, Iwenofu OH, Frankel WL, and Hitchcock CL (2010). Comparative mutational profiling in the assessment of lung lesions: should it be the standard of care? *Ann Thorac Surg* **90**, 388–396.

[37] Martini N, Bains MS, Burt ME, Zakowski MF, McCormack P, Rusch VW, and Ginsberg RJ (1995). Incidence of local recurrence and second primary tumors in resected stage I lung cancer. *J Thorac Cardiovasc Surg* **109**, 120–129.

[38] Aziz TM, Saad RA, Glasser J, Jilaihawi AN, and Prakash D (2002). The management of second primary lung cancers. A single centre experience in 15 years. *Eur J Cardiothorac Surg* **21**, 527–533.

[39] Motoi N, Szoke J, Riely GJ, Seshan VE, Kris MG, Rusch VW, Gerald WL, and Travis WD (2008). Lung adenocarcinoma: modification of the 2004 WHO mixed subtype to include the major histologic subtype suggests correlations between papillary and micropapillary adenocarcinoma subtypes EGFR mutations and gene expression analysis. *Am J Surg Pathol* **32**, 810–827.

[40] De Oliveira Duarte Achcar R, Nikiforova MN, and Yousem SA (2009). Micropapillary lung adenocarcinoma: EGFR, K-ras and BRAF mutational profile. *Am J Clin Pathol* **131**, 694–700.

[41] Nakamura Y, Niki T, Goto A, Morikawa T, Miyazawa K, Nakajima J, and Fukayama M (2007). c-Met activation in lung adenocarcinoma tissues: an immunohistochemical analysis. *Cancer Sci* **98**, 1006–1013.

[42] Kim YH, Ishii G, Goto K, Nagai K, Tsuta K, Shiono S, Nitadori J, Kodama T, Nishiwaki Y, and Ochiai A (2004). Dominant papillary subtype is a significant predictor of the response to gefitinib in adenocarcinoma of the lung. *Clin Cancer Res* **10**, 7311–7317.

[43] Ding L, Getz G, Wheeler DA, Mardis ER, McLellan MD, Cibulskis K, Sougnez C, Greulich H, Muzny DM, and Morgan MB, et al (2008). Somatic mutations affect key pathways in lung adenocarcinoma. *Nature* **455**, 1069–1075.

[44] Shedden K, Taylor JM, Enkemann SA, Tsao MS, Yeatman TJ, Gerald WL, Eschrich S, Jurisica I, Giordano TJ, and Misek DE, et al (2008). Gene expression-based survival prediction in lung adenocarcinoma: a multi-site, blinded validation study. *Nat Med* **14**, 822–827.

[45] Sholl LM, Yeap BY, Iafrate AJ, Holmes-Tisch AJ, Chou YP, Wu MT, Goan YG, Su L, Benedettini E, and Yu J, et al (2009). Lung adenocarcinoma with EGFR amplification has distinct clinicopathologic and molecular features in never-smokers. *Cancer Res* **69**, 8341–8348.

[46] Dacic S, Shuai Y, Yousem S, Ohori P, and Nikiforova M (2010). Clinicopathological predictors of EGFR/KRAS mutational status in primary lung adenocarcinomas. *Mod Pathol* **23**, 159–168.

[47] Huang J, Behrens C, Wistuba I, Gazdar AF, and Jagirdar J (2001). Molecular analysis of synchronous and metachronous tumors of the lung: impact on management and prognosis. *Ann Diagn Pathol* **5**, 321–329.

[48] Vansteenkiste JF, De Belie B, Deneffe GJ, Demedts MG, De Leyn PR, Van Raemdonck DE, and Lerut TE (2001). Practical approach to patients presenting with multiple synchronous suspect lung lesions: a reflection on the current TNM classification based on 54 cases with complete follow-up. *Lung Cancer* **34**, 169–175.

[49] Yoshino I, Nakanishi R, Osaki T, Hasuda S, Taga S, Takenoyama M, Yoshimatsu T, and Yasumoto K (1997). Postoperative prognosis in patients with non-small cell lung cancer with synchronous ipsilateral intrapulmonary metastasis. *Ann Thorac Surg* **64**, 809–813.

[50] Schneider F, Derrick V, Davison JM, Strollo D, Incharoen P, and Dacic S (2016). Morphological and molecular approach to synchronous non-small cell lung carcinomas: impact on staging. *Mod Pathol* **29**, 735–742.

[51] Riely GJ, Kris MG, Rosenbaum D, Marks J, Li A, Chitale DA, Nafa K, Riedel ER, Hsu M, and Pao W, et al (2008). Frequency and distinctive spectrum of KRAS mutations in never smokers with lung adenocarcinoma. *Clin Cancer Res* **14**, 5731–5734.

[52] Murphy SJ, Aubry MC, Harris FR, Halling GC, Johnson SH, Terra S, Drucker TM, Asiedu MK, Kipp BR, and Yi ES, et al (2014). Identification of independent primary tumors and intrapulmonary metastases using DNA rearrangements in non–small-cell lung cancer. *J Clin Oncol* **32**, 4050–4058.