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Clinical Implications of the New IASLC/ATS/ERS Classification of Lung Cancer

By A. Valeria Arrossi, MD, and Carol Farver, MD

Major advances in the understanding of the biology of lung non-small cell carcinomas (NSCLC) in the last decade opened the door to individualized therapeutic options for patients with lung cancer. In particular, the discovery of two targetable genetic alterations, the presence of mutations in the epidermal growth factor receptor (EGFR) gene and translocation of the ALK gene, conveyed a tremendous change in the managing of patients with lung cancer.

EGFR mutations are strong predictors of efficacy for treatment with inhibitors of the EGFR tyrosine kinase (EGFR-TKI), erlotinib and gefitinib. Clinical trials including patients with advanced EGFR mutant tumors have demonstrated longer progression-free survival when treated with EGFR-TKI therapy compared to those treated with standard chemotherapy.[1-3] In addition, response rate and outcomes are decreased in patients with EGFR wild-type tumors treated with EGFR TKIs.

Similarly, the presence of the translocation of the anaplastic lymphoma kinase (ALK) gene resulting in the creation of the equinoderm microtubule associated protein-like 4 (EML4)-ALK fusion transcript in lung adenocarcinomas resulted in greater response rates and progression-free survival when treated with the ALK inhibitor crizotinib.[4-5] Finally, several clinico-pathologic studies of adenocarcinomas have demonstrated that the presence and amount of certain histologic growth patterns are associated with overall outcomes.[6-8]

To reflect these advances, discoveries and observations regarding lung NSCLC, a new international multidisciplinary classification of adenocarcinomas has been published as a joint effort by the International Association for the Study in Lung Cancer (IASLC), the American Thoracic Society (ATS) and European Respiratory Society (ERS)[9], followed by the publications of clinical guidelines for the diagnosis of adeno-carcinoma in resection specimens and small specimens by the College of American Pathologists (CAP), IASLC and Association for Molecular Pathology (AMP).[10-11] Guidelines for the molecular diagnosis of NSCLC were independently published by the CAP and incorporated into the National Comprehensive Cancer Network (NCCN) guidelines.[12-13]

In general, these recommendations include three major changes. First, bronchioloalveolar cell carcinoma (BAC) is a confusing term that has gone through various interpretations and pathologic definitions over the past 20 years. Thus, a new term, lepidic growth pattern, has been recommended to replace BAC.

Second, several studies have shown that patients with small adenocarcinomas (less than 2 to 3.0 cm) with pure lepidic growth pattern or adenocarcinomas with focal non-lepidic growth pattern have 100% survival and near 100% survival at five years, respectively, if entirely resected.[6-8] These observations led to changes in the classification of these lepidic growth predominant adenocarcinomas, formerly known as “BAC” in resection specimens as follows[14]:

- A tumor less than 3.0 cm and shows pure lepidic growth pattern is referred to as adenocarcinoma in situ (AIS).
- A tumor that is less than 3.0 cm and shows non-lepidic growth pattern of no more than 5 mm is referred to as minimally invasive adenocarcinoma (MIA).
- If a non-lepidic growth pattern is present in an area of over 5 mm, (e.g. acinar, papillary, solid, etc) the tumor is referred to as an invasive adenocarcinoma.

AIS, MIA and invasive adenocarcinomas may be non-mucinous or mucinous, depending on cytological characteristics.

Important implications of this new classification affect staging and revision of the terminology used by pathologists in small specimens or cytology. The identification of a preinvasive category for adenocarcinomas in the lung, similar to in situ carcinomas (squamous cell carcinoma or adenocarcinoma) of other organs, mandates additional pathologic T stages of TIS and TMIA. Also, the presence of AIS may suggest tumor synchronicity if seen in multiple tumors in the lung, as opposed to intrapulmonary metastatic lesions. Finally, since the diagnosis of AIS and MIA can only be made after histologic evaluation of the entire tumor, these terms should not be used in biopsies or small specimens. Adenocarcinoma with lepidic growth is the preferred term in these samples to reflect the possibility of a non-lepidic pattern of adenocarcinoma in the not-sampled tumor.

Differentiation of adenocarcinoma versus squamous cell carcinoma is currently crucial for case selection for molecular testing of predictor biomarkers and therapy options, as pemetrexed, an anti-folate agent, and bevacizumab, a VEGF antibody, are only indicated in tumors with "non-squamous" histology. In addition, activating EGFR mutation and EML-ALK translocations are almost always present in NSCLC other than pure squamous cell carcinoma and are predictive of response to EGFR TKIs and ALK TKIs, respectively.

Therefore, providing more detailed information in those tumors that otherwise were formerly diagnosed as NSCLC in small biopsy or cytology specimens becomes an important step in the evaluation of biopsies/cytology with lung cancer. Tumors that have no morphologic evidence of squamous or adenocarcinoma differentiation should be further classified, in small specimens, based on IHC studies so that appropriate molecular testing is performed. However, given the small size of these specimens, IHC and histochemical work up should be limited to as few stains as possible to avoid tissue waste. These IHC panels include an adenocarcinoma marker (TTF-1, and/or Napsin-A) and a squamous cell carcinoma marker (p63, and/or p40).[15-16] Additional stains may be used for undetermined or equivocal cases with the 2-marker panel. Mucin stains may help to find intra-cytoplasmic mucin. NSCLC, NOS should be reserved for those cases where the NSCLCs has no definitive morphologic evidence of adenocarcinoma or squamous carcinoma, and either histochemistry and/or IHC show equivocal stain patterns, or the biopsy contains scant tissue for evaluation.

Based on these parameters, the new classification and guidelines for NSCLC diagnosis in small biopsies or cytology specimens are as follows[10-11]:

- Adenocarcinoma
- Squamous cell carcinoma
- NSCLC favor adenocarcinoma
- NSCLC favor squamous cell carcinoma
- NSCLC, not otherwise specified (NOS)

Finally, the CAP guidelines for the molecular diagnosis of NSCLC are summarized as follows:[12]

- EGFR mutation analysis and identification of ALK translocation by fluorescent in situ hybridization should be performed in all cases of advanced NSCLCs without evidence of pure squamous cell carcinoma.
- Clinical parameters should not be used for case-selection for molecular testing except in rare exceptions such as in young patients, never-smokers with biopsies demonstrating squamous cell carcinoma or small cell carcinoma.
- Molecular results should be available in no more than two weeks.

One of the most important implications that result from the new classification and guidelines resides in the approach to care of patients with lung cancer. The evaluation, diagnosis and treatment of patients with NSCLC should be based, nowadays, on the integration and intercommunication of a multidisciplinary team that includes pulmonologists, radio/oncologists, surgeons, radiologists and pathologists. The decision regarding the optimal approach to obtaining and processing diagnostic procedures that will provide specimens with adequate tumor tissue and/or cellularity for the appropriate and expeditious pathologic and molecular evaluations should be the result of discussions among the members of the multidisciplinary team. Similarly, the algorithms of molecular testing should be determined by the multidisciplinary team of each institution.

In summary, in resection specimens, adenocarcinomas with lepidic growth pattern should be separated into AIS, MIA or invasive adenocarcinoma based on size and lepidic growth pattern content. The distinction between histologic types of NSCLC in patients with lung cancer is critical for the selection of molecular testing and therapy choices in patients with NSCLC. Pathologic evaluation of lung cancer specimens is not always limited to morphologic evaluation, since limited ancillary stains, if appropriate, and eventual molecular testing, should be applied for proper patient care. Triaging the tissue maximizes the amount of tissue available for molecular studies.

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EGFR Mutation Analysis by Allele-Specific PCR

By Raymond R. Tubbs, DO, and Michael J. McNamara, MD

Cancers of the lung are aggressive diseases, accounting for approximately 30% of all cancer deaths in the United States in 2009, with the overall survival rate of patients with metastatic disease less than 15%.[1]

Different histological subtypes exist; Small-Cell Lung Cancer (SCLC), which accounts for approximately 20% of lung cancer cases, and Non-Small-Cell Lung Cancer (NSCLC), which accounts for the majority of lung cancer cases in the United States. NSCLC includes adenocarcinoma, squamous cell carcinoma and large-cell carcinoma. NSCLC tumors are also categorized according to molecular criteria. EGFR- (epidermal growth factor receptor) mutant NSCLC has been defined as a distinct, clinically relevant subset of lung cancer. EGFR-mutant tumors are histologically similar to adenocarcinomas, and are often associated with better prognosis than EGFR wild-type tumors, due in the most part to their increased sensitivity to Tyrosine Kinase Inhibitors (TKIs) such as erlotinib (Tarceva; Genentech/OSI Pharmaceuticals) or gefitinib (Iressa; AstraZeneca). However, not all mutations in the EGFR gene confer sensitivity to TKIs, and primary resistance is still observed. In addition to this, acquired resistance can develop after prolonged TKI exposure, which limits the effectiveness of this type of treatment.

EGFR is a transmembrane receptor belonging to the ERBB family of receptor tyrosine kinases, which also includes HER2, HER3 and HER4. After ligand binding, the EGFR receptor forms a dimer that activates receptor autophosphorylation through tyrosine kinase activity, which triggers a series of intracellular pathways that may result in cancer-cell proliferation, blocking apoptosis, activating invasion and metastasis, and stimulating tumor-induced neovascularization.[2,6] Small-molecule EGFR TKIs inhibit EGFR autophosphorylation and downstream signaling by reversibly competing with ATP to bind to the intracellular catalytic domain of EGFR tyrosine kinase.[2]

Activating mutations in EGFR occur in exons 18 to 21, which encode the kinase domain. These mutations are usually heterozygous, and amplification of the mutant allele can also occur.[1] The most common activating mutations found in EGFR are deletions in exon 19 centered around four amino acids (LREA) at positions 747 – 750, and a missense mutation (L858R) arising as a result of the leucine to arginine amino acid substitution at position 858 within exon 21. Together, these account for approximately 90% of TKI-sensitive mutations within the EGFR-mutant tumors.[1,7] The L861Q mutation in exon 21 and the substitution of glycine at position 719 in exon 18 with alanine (G719A), serine (G719S) or cysteine (G719C) also confer increased sensitivity to EGFR TKIs,[7,8] although these are much less common.

Together, the activating mutations discussed above are associated with response rates of approximately 70% when treated with erlotinib or gefitinib.[9] Analysis of the crystal structures of L858R and G719S EGFR mutants has demonstrated that the kinase is activated through disruption of autoinhibitory interactions, resulting in receptors with 50-fold more activity compared to wild-type EGFR receptors.[1] Recent studies have shown that patients with tumors harboring exon 19 deletions are associated with longer time to progression (TTP) and overall survival (OS) when compared with L858R point mutations,[9] presumably due to greater sensitivity to TKI treatments.

Mutations in exons 18 to 21 can also be associated with decreased sensitivity/primary resistance to TKIs. Small insertions or duplications in exon 20 (including D770_N771insG) have been shown to be less sensitive to TKIs than exon 19 deletion and L858R mutants. In fact, most patients with these mutations show progressive disease while undergoing TKI therapy.[1]

Acquired resistance can also arise, and despite an initial response to EGFR TKI therapy, patients with EGFR mutations rarely achieve a complete radiographic or pathologic response. Prolonged TKI treatment provides a selective pressure for the development of tumor cells with acquired resistance to gefitinib or erlotinib.[8] The T790M mutation which arises as a result of the substitution of threonine to methionine at position 790 in exon 20 has been found in 50% of patients with EGFR-mutant tumors who develop acquired resistance to TKIs.[1,3,4,8] This phenomenon is also seen in chronic myelogenous leukemia (CML) cells harboring ABL translocations and gastrointestinal stromal tumor (GIST) cells harboring activating KIT mutations, whereby the threonine gatekeeper residues are substituted with bulkier isoleucine residues that alter drug binding in both ABL (T315I) and KIT (T670I).[1] The T790M mutation is rare, occurring in <5% of untreated

EGFR mutant tumors, but is detected as a second site mutation in half of patients who develop acquired resistance to EGFR TKI therapy.[1] Evidence exists to show that acquired resistance can be lost after a period without TKI therapy,[1,5] however the mechanisms involved in this are not yet fully understood, and a recommendation regarding discontinuation of EGFR TKI therapy after developing acquired resistance has not yet been made.

Numerous studies and trials have been conducted emphasizing the importance of using genomic assessments of EGFR rather than using clinical characteristics, as the former is more accurate in selecting a group of patients with increased chance of sensitivity to EGFR-TKI therapy.[9]

The Qiagen EGFR RGQ PCR assay uses Scorpions and ARMS® technologies to detect the following 29 somatic mutations (full details in Table 1) against a background of wild-type genomic DNA:

- 19 deletions in exon 19 (detects the presence of any of the 19 deletions, but does not distinguish between them)
- T790M
- L858R
- L861Q
- G719X (detects the presence of any of Q719S, G719A or G719C, but does not distinguish between them)
- S768I
- 3 insertions in exon 20 (detects the presence of any of 3 insertions but does not distinguish between them).

Table 1: Mutations detected by the EGFR Rotor-Gene Q Allele Specific PCR assay

Exon	Mutation	in NSCLC, response to EGFR TKI therapy, caused by this mutation	References	COSMIC ID	Base Change (CDS Mutation)	Amino Acid Mutation
18	G719A	sensitive to EGFR TKI treatment	1,7,8	6239	c.2156G>C	p.G719A
	G719S			6252	c.2155>A	p.G719S
	G719C			6253	c.2155G>T	p.G719C
19	Deletions in exon 19	sensitive to EGFR TKI treatment	1,7,9	6223	c.2235_2249del15	p. E746_A750del
				13551	c.2235_2252>AAT (complex)	p. E746_T751>I
				12728	c.2236_2253del18	p.E746_T751del
				12678	c.2237_2251del15	p.E746_T751>A
				12367	c.2237_2254del18	p.E746_S752>A
				12384	c.2237_2255>T (complex)	p.E746_S752>V
				6225	c.2236_2250del15	p.E746_A750del
				6220	c.2238_2255del18	p.E746_S752>D
				12422	c.2238_2248>GC (complex)	p.L747_A750>P
				12419	c.2238_2252>GCA (complex)	p.L747_T751>Q
				6218	c.2239_2247del9	p.L747_E749del
				6254	c.2239_2253del15	p.L747_T751del
				6255	c.2239_2256del18	p.L747_S752del
				12382	c.2239_2248TTAAGAGAAG>C (complex)	p.L747_A750>P
				12387	c.2239_2258>CA (complex)	p.L747_P753>Q
				6210	c.2240_2251del12	p.L747_T751>S
				12370	c.2240_2257del18	p.L747_P753>S
				12369	c.2240_2254del15	p.L747_T751del
				12383	c.2239_2251>C (complex)	p.L747_T751>P
20	Insertions in exon 20	lack of responsiveness to EGFR TKIs	1	12376	c.2307_2308ins9	p.V769_D770insASV
				12377	c.2319_2320insCAC	p.H773_V774insH
				12378	c.2310_2311insGGT	p.D770_N771insG
20	T790M	resistance to EGFR TKIs	1,3,4,5,8	6240	c.2369C>T	p.T790M
20	S768I	low level sensitivity to EGFR TKI treatment	7	6241	c.2303G>T	p.S7681
21	L858R	sensitive to EGFR TKI treatment	1,7	6224	c.2573T>G	p.L858R
21	L861Q	sensitive to EGFR TKI treatment	7,8	6213	c.2582T>A	p.L861Q

The ARMS® (Amplification Refractory Mutation System) technology achieves allele- or mutation-specific amplification. Taq DNA polymerase is effective at distinguishing between a match and a mismatch at the 3' end of a PCR primer. Specific mutated sequences are selectively amplified, even in samples where the majority of the sequences do not carry the mutation. When the primer is fully matched, the amplification proceeds with full efficiency. When the 3' base is mismatched, only low level background amplification occurs.[10]

Scorpions, or bi-functional molecules containing a PCR primer covalently linked to a probe, are used to detect amplification. The fluorophore in this probe interacts with a quencher, also incorporated into the probe, which reduces fluorescence. During PCR, when the probe binds to the amplicon, the fluorophore and quencher become separated. This leads to an increase in fluorescence from the reaction tube.[10] A total of seven mutation reactions, each labeled with FAM are performed. Each mutation reaction contains Scorpion probes plus primers for discrimination between the wild-type DNA and mutant DNA detected by real time PCR.

A control reaction (a region of exon 2 for which the primers and probe have been designed to avoid any known EGFR polymorphisms), also labeled in FAM, is performed to assess the amount of amplifiable DNA in the sample and to calculate the difference in cycle threshold (ΔC_T) between the mutation reaction and the control reaction from the same sample.

A sample is considered mutation positive if the ΔCT is less than the cut-off ΔCT for that mutation reaction. Above this value, the sample may either contain less than the percentage of mutant DNA detectable by the assay, or the sample does not contain that specific mutation. The assay is capable of detecting mutations in samples containing at least 10% mutant allele. This is representative of 20% tumor within a heterogeneous sample containing tumor and non-tumor cells.

When using ARMS primers, some inefficient priming may occur, giving a very late background CT from DNA not containing a mutation. All CT values calculated from background amplification will be greater than the cut-off ΔCT values and the samples will be classed as mutation negative.[10]

This is particularly noticeable in the T790M mutation reaction, due to the fact that the T790M mutation is found in a very GC rich region. With primer design, GC content is critical for PCR efficiency. The sensitivity of the T790M assay is affected due to an increased limit of blank (LOB), which only allows an increased % of mutant to be discriminately detected. In the assay, this reflects itself in a lower ΔCT cut-off, and the potential for 'breakthrough' (non-specific) amplification late in the reaction.

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Genotyping for ALK Rearrangements in Liquid-Based Cytopathology Specimens: ThinPrep FISH

By Raymond R. Tubbs, DO, and Jordan P. Reynolds, MD

Lung cancer is the second most common malignancy overall in the United States and despite improved detection and treatment, remains the leading cause of cancer-related death in men and women worldwide.[1] Lung cancers have been classically divided into small cell and non-small cell types based on morphology and clinical features. With recent advances in molecular pathogenesis and the discovery of specific targetable genetic alterations in certain lung carcinomas, this division has become essential for therapeutic management.

Non–small cell lung carcinomas (NSCLC) represent approximately 80% of lung cancers and are further classified into adenocarcinoma (40%-50%), squamous cell carcinoma (30%) and large cell carcinoma (9%). A subset of NSCLC, mostly adenocarcinomas, exhibit mutations of epidermal growth factor receptors (EGFR) that confer sensitivity to tyrosine kinase inhibitors. Targeted therapies with such inhibitors have been shown to improve the otherwise dismal survival in patients with tumors harboring EGFR activating mutations.[2]

The anaplastic lymphoma kinase (*ALK*) gene encodes a receptor tyrosine kinase that is normally not expressed in lung cells. Approximately 5% of NSCLC harbor *ALK* gene rearrangements, most commonly in the form of a small intrachromosomal inversion, inv(2)(p21;p23), with the fusion of *ALK with echinoderm microtubule associated protein like 4* (*EML4*) gene.[3] The ALK/EML4 fusion produces an abnormal, constitutively active chimeric protein kinase with oncogenic properties.[4,5] Targeted ALK tyrosine kinase inhibitors have proven anticancer activity, with crizotinib showing a good clinical response in advanced NSCLC patients harboring *ALK* rearrangements.[6]

While relatively uncommon in NSCLC, the presence of *ALK* rearrangements has major therapeutic implications, and thus needs to be assessed through an accurate, reproducible and accessible diagnostic test. The U.S. Food and Drug Administration approved an *in-vitro* diagnostic class fluorescence in-situ hybridization (FISH) test as a companion diagnostic tool for crizotinib-based treatment eligibility [Abbott Molecular Vysis (AMV)]. As a consequence, FISH is currently considered the gold standard for ALK-status testing and it can be performed on formalin-fixed paraffin-embedded (FFPE) material. The commercial break-apart FISH format consists of red and green probes that flank the highly conserved translocation breakpoint within the *ALK* gene. Normal cells exhibit yellow fusion signals while neoplastic cells harboring ALK rearrangements show split red and green signals. A case is interpreted as ALK-positive by FISH when 15% or more tumor cell nuclei demonstrate isolated green and red or isolated red signals among 50 tumor nuclei scored.[7]

With the current emphasis on minimally invasive diagnostic procedures, testing for ALK rearrangements by FISH is increasingly attempted on small specimens, including biopsies or FFPE-cytoblocks prepared from fine needle biopsy (FNA) samples. In certain cases, like those of patients with late-stage disease, small FNA specimens may be the only material available, as these patients are not eligible for surgery.

At our institution, the ThinPrep liquid based cytology platform (Hologic, Bedford, MA) is preferred for processing lung FNA specimens due to several advantages including: minimal artifacts, air-drying, lack of smear-crushing and homogenous cell enrichment with nearly 100% cell transfer to slide.[8] The protocol involves generation of a cell pellet from the FNA sample collected in CytoLyt, which is then divided for the preparation of a ThinPrep slide used for diagnosis and of a cytoblock used for molecular testing.[9] The material obtained from an FNA procedure may not be sufficient for FFPE-cytoblock preparation and further molecular testing. Even more frequently in our experience, a significant proportion of

Table 1. Breakdown of available and informative NSCLC samples by type and test.

Sample Type	Total Available	Informative by ThinPrep-FISH	Informative by IHC	Informative by ThinPrep-FISH and IHC
ThinPrep	230	228	N/A	**154**
Cytoblock	211	N/A	154	

Table 2. Correlation between ThinPrep-FISH and D5F3-IHC on 154 available dual-informative NSCLC samples.

		D5F3-IHC		
		Positive	Negative	Total
ThinPrep-FISH	Positive	10	2	
	Negative	0	142	
				154
Sensitivity		100%	(95% CI: 0.65-1.00)	
Specificity		98.6%	(95% CI: 0.94-0.99)	
Positive predictive value		83.3%	(95% CI: 0.50-0.97)	
Negative predictive value		100%	(95% CI: 0.96-1.00)	
Overall agreement		**98.7%**		

the cytoblocks contain few if any recognizable tumor cells, despite the presence of a seemingly adequate initial cell pellet and of abundant tumor cells on the ThinPrep slide used for diagnosis. This issue has also been reported by other laboratories[10] and has been attributed to the inherent variability in the cytoblock preparation process which involves human handling. Inadequacy of cytoblocks for molecular testing is a problematic issue that can lead to treatment delays in a subset of cases and result in repeat procedures necessary to obtain additional material.

Our study of 230 FNA samples of lung tissue included samples obtained by transbronchial needle biopsy, bronchial brush, pleural and pericardial fluid from 217 patients with advanced NSCLC seen at Cleveland Clinic. This also included a consecutive series of 179 samples collected from 174 patients between December 2012 and August 2013. Here we demonstrated on a large cohort that ALK-FISH testing can successfully be performed directly on ThinPrep liquid based cytology slides. Using ThinPrep slides over direct smears for ALK-FISH testing can be advantageous for many of the reasons liquid based preparations are preferred for cytology diagnosis purposes. More importantly, several advantages can be envisioned for performing ALK-FISH directly on ThinPrep slides as compared to slides derived from FFPE cytoblocks. In addition to the already mentioned superior adequacy for sufficient tumor compared to FFPE tissue sections, cytologic preparations such as ThinPrep allow assessment of the entire tumor cell nucleus, thus avoiding signal loss by section artifacts or incomplete penetration of probes into the tissue, and providing more accurate signal counts. Furthermore, the etching of areas with abundant tumor directly on the ThinPrep slides at the time of diagnosis allows precise FISH probe enumeration in tumor cells without interference from normal cells. Indeed, consistent with the previous report,[11] we observed robust FISH signals on the

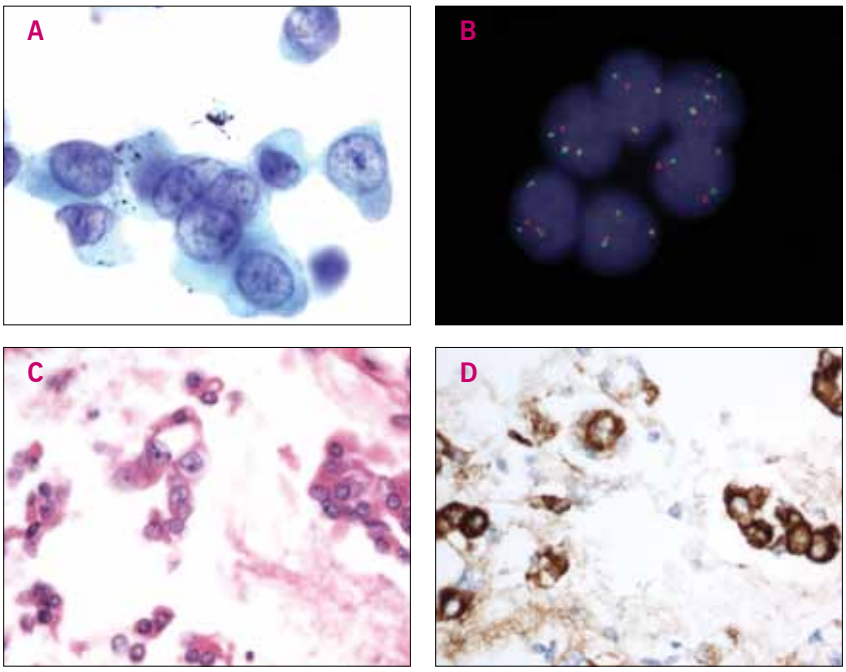

Figure 1. Representative images of NSCLC positive for ALK rearrangements by ThinPrep-FISH showing a typical positive pattern (A, B 1000x), and positive for ALK expression by D5F3-IHC on the cytoblock (C, H&E 400x; D, D5F3-IHC, 400x).

ThinPrep slides, with representation of the both described patterns (separated green and red signals as well as isolated red signals) in the positive cases.

Our consecutive case subseries included 9 NSCLC specimens positive for ALK rearrangements by ThinPrep-FISH. The percentage of ALK-positive samples in our study (9/179, 5% in the consecutive series; 18/228, 7.8% overall) was within the cited range (2-13%).[3] The increased proportion of positive cases overall compared to the consecutive subseries is likely due to the fact that multiple unique specimens were analyzed for several patients with ALK-positive tumors biopsied before December 2012.

Due to the high failure of ALK-FISH on cytoblocks, we used ultrasensitive immunohistochemistry (IHC) as a reference. We previously demonstrated that ultrasensitive D5F3 ALK-IHC for ALK has nearly perfect correlation with ALK-FISH and is informative in cases with limited tumor cells on the FFPE cytoblocks that are otherwise inadequate for FISH testing. In the current study, ThinPrep-FISH showed a high correlation with ALK-IHC in assessing ALK status, with 100% sensitivity and 98.6% specificity.

In our study, there were significantly fewer uninformative ThinPrep-FISH results due to sample quality or quantity when compared to ALK-IHC (0.8% vs 27%, $p<0.001$). Furthermore, within 74 samples with uninformative ALK-IHC and successful ThinPrep-FISH, the latter test was positive in 6 cases (8.1%).

This data indicates that ThinPrep-FISH can be successfully utilized on cytology samples that are limited or suboptimal for cytoblock analysis by FFPE-FISH or IHC, and can effectively detect a subset of positive cases that would be otherwise missed by FFPE-FISH or IHC analysis alone. Taken together, our data demonstrates that ThinPrep-FISH represents a reliable method for initial ALK testing in NSCLC.

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About the Authors

Jordan P. Reynolds, MD

Jordan Reynolds, MD, joined Cleveland Clinic in 2011 as a staff pathologist in the departments of Anatomic Pathology and Molecular Pathology in the Pathology and Laboratory Medicine Institute.

Dr. Reynolds served fellowships in cytopathology and surgical and medical pathology at the Mayo Graduate School of Medicine at Mayo Clinic. He completed his residency in anatomic and clinical pathology at the University of Cincinnati, where he also served as chief resident in the Department of Pathology and Laboratory Medicine.

Dr. Reynolds received his medical degree from Northeastern Ohio Universities College of Medicine and his bachelor of science degree from Kent State University. He is a Diplomate of the American Board of Pathology in anatomic and clinical pathology, and cytopathology. He also is a member of the National Medical Association, American Society of Cytopathology, and the United States and Canadian Academy of Pathology.

Dr. Reynolds has contributed to numerous publications and scientific presentations, and is the recipient of several awards. His interests are cytology with an emphasis on thyroid and urines, and genitourinary pathology.

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Raymond R. Tubbs, DO

Please refer to page 9 for Dr. Raymond R. Tubbs' complete bio.

Allogen Laboratories Joins Pathology & Laboratory Medicine Institute

Allogen Laboratories, one of the largest and most sophisticated independent, full-service, histocompatibility, immunogenetics and transplant immunology laboratories in the United States, is joining Cleveland Clinic's Robert J. Tomsich Pathology & Laboratory Medicine Institute (RT-PLMI) as a center within RT-PLMI.

"Allogen Laboratories share our mission and vision of dedication to clinical excellence, innovation, education and research," said Kandice Kottke-Marchant, MD, PhD, chair of RT-PLMI, in making the announcement on February 21. "This alignment will empower Allogen to leverage the extensive expertise and resources of RT-PLMI to maximize its growth potential and further its leadership in the field worldwide."

As part of the transition, Dan Kubiak, Continuous Improvement Director from RT-PLMI, will join the Allogen Laboratories' team as the administrative lead and Director of Allogen. In this role he will serve as a key partner to Medhat Askar, MD, PhD, on working to build on the mission and shape the future of Allogen.

Medhat Askar, MD, PhD; Kandice Kottke-Marchant, MD, PhD; Dan Kubiak.

Kubiak, who has a strong foundation in operations and performance improvement, will continue in his role as Continuous Improvement Director as he takes on new responsibilities with Allogen.


Deborah Chute, MD


Tarik Elsheikh, MD


Carol Farver, MD


John R. Goldblum, MD


Rish Pai, MD, PhD


Brian Rubin, MD, PhD


Lisa Yerian, MD

RT-PLMI Staffers Contribute to Success of USCAP Meeting

The Robert J. Tomsich Pathology & Laboratory Medicine Institute (RT-PLMI) was again well represented at the 103rd annual meeting of USCAP, the world's largest pathology meeting, in the San Diego Convention Center February 28 through March 6.

"We are very proud of our residents, fellows and faculty members who contributed to the success of this highly prestigious meeting," said John R. Goldblum, MD, Chair of the Department of Anatomic Pathology.

Cleveland Clinic residents, fellows and faculty presented 74 abstracts at this meeting. In the majority of abstracts, RT-PLMI residents and fellows served as the first author for these outstanding presentations.

In addition to the strong presence in the poster and platform sessions, no other institution was as strongly represented in the large number of educational activities presented at this meeting. Four RT-PLMI faculty (Brian Rubin, MD, PhD; Rish Pai, MD, PhD; Lisa Yerian, MD; and Tarik Elsheikh, MD) served as short course directors, which is one of USCAP's primary educational forums. Three faculty members (Eric Hsi, MD; Deborah Chute, MD; and Carol Farver, MD) served as presenters for the Evening Subspecialty Conferences, and three faculty members (Jesse McKenney, MD; Roy Lee, MD; and Dr. Rubin) served as either moderators or presenters in the various Companion Society Meetings held at the front end of the meeting.

A number of faculty members served as scientific platform moderators, and three faculty served as course directors and/or presenters of USCAP Special Courses, including Dr. Farver (course director and presenter for the Residents Workshop: Leadership, Collaboration and Change in Healthcare — A Resident's Workshop for Essential Skills). Similarly, Dr. Elsheikh served as course director and presenter for the Special Course entitled "Basic Principles in Cytology." Fadi Abdul-Karim, MD, also served as a presenter at this day-long course. Dr. Goldblum served as the moderator for the inaugural Hot Topics in Gastrointestinal Pathology luncheon meeting.

Several individuals also hold key positions with the USCAP or related companion societies, including Dr. Goldblum (Vice President of USCAP Executive Committee, member of the Innovative Educational Products Committee [IEPC]). Dr. McKenney serves as the Short Course Coordinator for the USCAP Education Committee, and Dr. Billings is also a member of this important committee. Dr. Billings also served as a member of the USCAP Subcommittee for Unique Live Course Offerings, and Drs. Elsheikh and Pai are members of the IEPC. One of our residents, Trent Marberger, MD, serves as a member of the USCAP Resident Advisory Committee, Deborah Chute, MD, is a member of the important USCAP Stowell-Orbison Award Committee, and Dr. Elsheikh also serves as chair of the USCAP Foundation.

Finally, Dr. Rubin has completed his tenure as one of the senior editors for one of the two official USCAP journals, *Laboratory Investigation*.

Remembering Raymond Tubbs, DO

It is with a heavy heart that I announce the passing of RT-PLMI's beloved Ray Tubbs, DO. Dr. Tubbs passed away on Saturday, April 19, after battling cancer. Dr. Tubbs was a true innovator and leader, having served as staff in the Robert J. Tomsich Pathology & Laboratory Medicine Institute for more than 35 years. He truly impacted not only Cleveland Clinic, but the national and international pathology and laboratory medicine community. We will be forever inspired by his contributions to hematopathology, molecular oncology and molecular pathology.

Dr. Tubbs earned his doctorate at Kirksville College of Osteopathic Medicine in 1973. He then came to Cleveland Clinic for internship and residency training in Internal Medicine. He completed an additional residency in Anatomic & Clinical Pathology in 1978 and joined the staff in Clinical Pathology. He later added a joint appointment in Cancer Biology in the Lerner Research Institute. He served as Chairman of the Department of Clinical Pathology from 1997-2007, and was a Professor of Pathology in the Lerner College of Medicine until his death.

Dr. Tubbs published more than 600 peer-reviewed articles, reflecting his relentless pursuit of new ideas and discoveries that might make a difference for patients. He co-authored two of the articles featured in this issue of Pathology Innovations. Many of the diagnostic tests he pioneered are now standard practice.

Those who worked with him or were mentored by him will continue to be inspired by his focused work ethic, love of learning and integrity.

Kandice Kottke-Marchant, MD, PhD
Chair, Robert J. Tomsich Pathology & Laboratory Medicine Institute

Pathology Innovations Magazine offers information from the medical staff in the Cleveland Clinic's Robert J. Tomsich Pathology & Laboratory Medicine Institute about its research, services and laboratory technology.

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Check out our redesigned website for the latest information on Cleveland Clinic Laboratories. Here you will find our test directory, technical briefs, technical updates, policies and procedures, specimen collection centers and contact information.

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