Review Article

# Pulmonary adenocarcinoma: implications of the recent advances in molecular biology, treatment and the IASLC/ATS/ERS classification

**Swaroop Revannasiddaiah[1], Priyanka Thakur[2], Bhaskar Bhardwaj[3], Sridhar Papaiah Susheela[4], Irappa Madabhavi[5]**

[1]Department of Radiation Oncology, Swami Rama Cancer, Hospital & Research Institute, Government Medical College-Haldwani, Nainital, Uttarakhand, India; [2]Department of Radiotherapy, Regional Cancer Centre, Shimla, India, [3]Department of Pulmonary Medicine, Indira Gandhi Medical College, Shimla, India; [4]Department of Radiation Oncology, HealthCare Global-Bangalore Institute of Oncology, Bengaluru, Karnataka, India; [5]Department of Medical, Oncology, Gujarat Cancer Research Institute, Ahmedabad, Gujarat, India

*Correspondence to:* Swaroop Revannasiddaiah, MD. Department of Radiation Oncology, Swami Rama Cancer Hospital & Research Institute, Government Medical College-Haldwani, Nainital, Uttarakhand, India. Email: swarooptheone@gmail.com.

**Abstract:** A decade ago, lung cancer could conveniently be classified into two broad categories—either the small cell lung carcinoma (SCLC), or the non-small cell lung carcinoma (NSCLC), mainly to assist in further treatment related decision making. However, the understanding regarding the eligibility of adenocarcinoma histology for treatments with agents such as pemetrexed and bevacizumab made it a necessity for NSCLC to be classified into more specific sub-groups. Then, the availability of molecular targeted therapy with oral tyrosine kinase inhibitors (TKIs) such as gefitinib and erlotinib not only further emphasized the need for accurate sub-classification of lung cancer, but also heralded the important role of molecular profiling of lung adenocarcinomas. Given the remarkable advances in molecular biology, oncology and radiology, a need for felt for a revised classification for lung adenocarcinoma, since the existing World Health Organization (WHO) classification of lung cancer, published in the year 2004 was mainly a pathological system of classification. Thus, there was a combined effort by the International Association for the Study of Lung Cancer (IASLC), the American Thoracic Society (ATS) and the European Respiratory Society (ERS) with an effort to inculcate newly established perspectives from clinical, molecular and radiological aspects in evolving a modern classification for lung adenocarcinomas. This review provides a summary of the recent advances in molecular biology and molecular targeted therapy with respect to lung adenocarcinoma. Also, a brief summation of the salient recommendations provided in the IASLC/ATS/ERS classification of lung adenocarcinomas is provided. Lastly, a discussion regarding the future prospects with lung adenocarcinoma is included.

**Keywords:** Lung adenocarcinoma; pulmonary adenocarcinoma; IASLC/ATS/ERS classification; EGFR; EML4-ALK; erlotinib; gefitinib; crizotinib; tyrosine kinase inhibitors (TKIs); bronchioloalveolar carcinoma (BAC)

Submitted Jan 05, 2014. Accepted for publication May 16, 2014.
doi: 10.3978/j.issn.2072-1439.2014.05.19
**View this article at:** http://dx.doi.org/10.3978/j.issn.2072-1439.2014.05.19

## Introduction

The recent years have witnessed many a breakthrough in the management of various malignancies. These breakthroughs have been possible not only due to innovations in treatment methodologies, but also due to enhanced understanding of pathological, molecular and genetic basis of cancers. In this day and age, almost every malignancy is amenable for sub-classification, which has been possible due to an enhanced understanding of the heterogeneous nature of malignancies. These classifications help not only in prognostic stratification,

but also in guiding specific treatments.

Across the years, multiple new therapeutic agents have been made available for the management of lung cancer. However, this comes with a caveat—all agents cannot be applied in all patients of lung cancer. The histology dependence within non-small cell lung cancer (NSCLC) was demonstrated by the fact that the use of pemetrexed was associated with no benefits among patients with squamous cell histology (1). Additionally, it was understood that the use of bevacizumab was associated with risks of life-threatening hemorrhage when used in patients with squamous cell histology (2). Thus, the importance of sub-classifying 'NSCLC' into more specific histological subtypes is now very justifiable.

More than half of all NSCLC diagnosed worldwide happen to be adenocarcinomas (3). However, adenocarcinomas in themselves are not all the same, since a lot of heterogeneity exists in terms of pathological and molecular features. In the recent years, the runaway success had with the use of imatinib for molecular targeting of the mutated *BCR-ABL* gene led to the initiation of efforts by various research groups to develop targeted therapies for many malignancies, including lung cancer (4). While the pre-clinical studies with the use of tyrosine kinase inhibitors against mutated epidermal growth factor receptors (*EGFR*)-TKIs appeared to offer good prospects for the treatment of lung cancer, it was later understood via clinical trials that the benefit with *EGFR*-TKIs was not applicable to all patients of NSCLC. Sub-group analyses of initial phase-III trials implied better benefits among specific populations, such as those patients with adenocarcinoma histology, patients of East Asian origin, never-smokers, and women (5). This selective specificity of benefit with *EGFR*-TKIs fueled further efforts into molecular profiling, which revealed that the benefit with *EGFR*-TKIs was specific to patients with *EGFR* gene mutations, which in turn were more likely to be found in tumors with adenocarinoma histology. In addition to *EGFR*-targeting, recent understanding regarding various other driver mutations in lung adenocarcinomas has led to intense research efforts towards development of novel agents towards other mutations. Recent success has also been observed with the use of crizotinib, an agent which targets mutations involving *ALK*-gene mutations (6). However, beyond *EGFR* and *ALK* targeted approaches, the progress in targeting other known mutations has rather been minimal, and remains to be a focus of ongoing research.

The advances in management of lung adenocarcinoma have not necessarily been restricted to molecular breakthroughs, but are also attributable to advances in multiple other disciplines including pathology, radiology, oncology and surgery. The prior classification of lung cancer, which was developed by the World Health Organization (WHO) was mainly a pathological classification, developed by panels mainly consisting of pathologists (7). Thus, there was a need for a modern classification which would encompass multidisciplinary perspectives in the classification of lung adenocarcinomas. Working in this direction, there was a combined effort by the International Association for the Study of Lung Cancer (IASLC), the American Thoracic Society (ATS) and the European Respiratory Society (ERS). The joint effort by the IASLC, ATS and the ERS culminated in the development of comprehensive guidelines (published in the year 2011) for the classification of lung adenocarcinomas, which not only provided classification guidelines, but also provided guidance towards good practice and recommendations for further research (8).

In this review, the recent understandings with respect to the molecular biology and molecular targeted therapy in lung adenocarcinomas are discussed. Also, a concise summary of the important recommendations in the recent IASLC/ATS/ERS classification of lung adenocarcinoma is also provided. Lastly, potentially emerging issues which could affect the management protocols of lung adenocarcinomas in the coming years are also discussed.

## Molecular biology of lung adenocarcinoma

The 'hallmarks of cancer' imply abilities of limitless replicative potential, self sufficiency at growth, anti-apoptotic potential, sustained angiogenesis and the potential for invasion and metastasis. These mentioned 'abilities' are acquired due to dysregulation of signaling pathways. For a malignancy to occur, the underlying genetic mechanisms could include oncogene activation (via gene amplification, rearrangements, and point mutations), or via loss of tumor suppressor gene function (by loss of heterozygosity, or by epigenetic transcriptional silencing). Both these mechanisms have been understood to be involved in the etio-pathogenesis of lung cancer (9,10).

This section describes the recent advances in relation to the advances in molecular biology of oncogene activation leading to lung cancer. Though loss of tumor suppressor gene function as an etiology of lung cancer was understood much before the concept of oncogene activation was even postulated, there have been greater efforts towards understanding the molecular biology behind oncogene

 

activation, mainly owing to the feasibility of molecular targeted therapy.

Research into the mechanisms involved in oncogene activation led to the discovery of the phenomenon of 'oncogene addiction'. This recently described phenomenon states that certain tumors rely upon one single dominant oncogene for the purpose of initiation, growth and survival, and that the inhibition of this specific oncogene leads to the regression of the particular tumor. Proof for the concept can be had from the myriad examples of success had from molecular targeting, such as with the use of imatinib for the inhibition of the *BCR-ABL* fusion gene in chronic myeloid leukemia, or the use of traztuzumab for the treatment of human epidermal receptor 2 (*HER2)* over-expressing breast cancer (6,11,12).

The oncogenes involved in 'oncogene addiction' are also termed as 'driver oncogenes'. These driver oncogenes represent conditional vulnerability in lung cancer—given that the tumor cells are dependent on the aberrant gene function for survival and proliferation. These in a way represent the 'achilles heel' of the tumor, given that the phenomenon allows an unique therapeutic opportunity since the targeting of the driver oncogene would lead to specific killing of the 'oncogene addicted' tumor cells alone (13,14).

In lung cancer, the commonly activated driver oncogenes include *EGFR*, kirsten rat sarcoma oncogene (*KRAS*), *HER2*, *MYC*, mesenchymal epidermal transition (*MET*), *EML4-ALK* and *BCL2*. Clinically, *EGFR* mutations are the most important not only because of them being among the most common mutations in lung adenocarcinoma, but also because of the availability of effective targeted therapies against the same. The translocation mutation *EML4-ALK* too has garnered enough attention recently because of the availability of crizotinib for targeting. The clinical success in targeted therapies for lung adenocarcinoma has unfortunately for now been restricted to *EGFR* and *ALK* mutations, and intense research is however underway to develop novel agents to target other known driver mutations (15-18).

### *EGFR mutations in lung adenocarcinoma*

*EGFR* belongs to a family of trans-membrane receptor tyrosine kinases, consisting of three other closely related receptors—the *HER2*, *HER3*, and the *HER4*. The *EGFR* and other members of the family are expressed in various normal tissues of epithelial, mesenchymal and neural origin. Knockout studies in mice have demonstrated that *EGFR* receptor family is necessary for survival, and is involved in development and maintenance of important organs such as the skin, mucosa, heart, lungs and the central nervous system (19,20).

Given the pivotal role of *EGFR* in regulating growth, it is not surprising that mutations involving the *EGFR* are oncogenic. Small molecule *EFGR* inhibitors are inhibitors of tyrosine kinases, which block the binding of ATP to the tyrosine kinase catalytic domain. The success had with oral *EGFR* inhibitors has been remarkable enough for them to be approved for first line use in locally advanced NSCLC with *EGFR* mutated status.

The exact worldwide prevalence of *EGFR* mutations among adenocarcinoma patients is difficult to estimate. The main reason being that *EGFR* mutations' occurrence seems to vary with ethnicity- being very uncommon among blacks, with an incidence of <20% of all NSCLC among whites, and with an incidence of 20-40% of all NSCLC among patients of East Asian origin. It must however be noted that the available statistics mostly estimate the incidence among NSCLC as a whole, and it is only recently since the publication of the IASLC/ATS/ERS classification guidelines that the importance of sub-classifying NSCLC into more specific subtypes has been realized. Nevertheless, if a population of non-smokers, with adenocarcinoma histology, and Asian origin were to be considered, then the likelihood of the tumor harboring *EGFR* mutations would be well over 60% (5,21-23).

The mutations affecting the kinase domain region (located from exon 18 to 21) of the *EGFR* gene are regarded as 'activating mutations' since these mutations result in constitutive kinase activity of the receptor kinase (24). The most frequent of these activating mutations involve various mutations at exon 19 amounting for 45% of the cases, while point mutations (mostly the *L858R* mutation) account for 40% of the cases. The remaining 15% is comprised of various point or insertion mutations in exons 18 to 21 (25,26). There is evidence to state that differential sensitivity to TKIs among patients with regards to the type of *EGFR* mutation (27,28).

### *Targeting the mutant EGFR*

Prior to realization of the potential with *EGFR* inhibition, NSCLC was a 'clinical entity' amenable to treatment with cytotoxic chemotherapy, and this offered modest response rates of 25-30% and a median survival (MS) of about a year (29). In 2004, three independent research groups reported that

 

*EGFR* mutations in lung cancer were amenable for targeting by TKIs (30-32).

Initial studies testing the efficacy of *EGFR*-TKIs had involved NSCLC patients after prior treatment with chemotherapy. These early studies such as the BR.21 and the INTEREST trials were conducted in unselected patients of NSCLC without regards to their *EGFR* mutation status. Despite the fact that these trials involved both mutated and non-mutated patients, there was evidence of benefit with the use of gefitinib/erlotinib in comparison to placebo or chemotherapy. The BR.21 trial involved 731 previously treated NSCLC (unselected mutation status) allotted to treatment with erlotinib versus placebo. Erlotinib was better in terms of both progression free survival (PFS) (2.2 *vs.* 1.8 months, P<0.001) and MS (6.7 *vs.* 4.7 months, P<0.001) (33). The phase III trial included 1,433 previously treated NSCLC patients (not selected as per *EGFR* INTEREST status). The study compared second line treatment with gefitinib versus docetaxel. The study indicated non-inferiority of gefitinib to docetaxel (34).

Though the BR.21 and the INTEREST trials used second line treatment with *EGFR*-TKIs in NSCLC patients untested for *EGFR* mutations, there were many studies which did not support this approach in comparison to the use of second line chemotherapy. The phase-III trial V-15-32 was conducted in Japan to assess the efficacy of second line treatment with gefitinib in comparison to second line chemotherapy with docetaxel. This study failed to show non-inferiority of gefitinib to docetaxel among NSCLC patients unselected as per histology or *EGFR* status (35).

From sub-group analysis of previous trials, reports were made that *EGFR*-TKIs were of greater effect in certain populations—such as in patients of Asian origin, non-smokers and those with adenocarcinoma histology. Thus, trials were then on designed based on patients selected by background characteristics. The IPASS trial was conducted with an intention to assess the efficacy of first-line treatment with gefitinib in previously untreated Asian non-smokers with advanced adenocarcinoma. The study involving 1,217 patients compared gefitinib versus chemotherapy with carboplatin-paclitaxel. Though the overall PFS was better with gefitinib, there was controversy in interpretation since the survival curves of the two groups crossed each other. Thus, it was concluded that for the entire study population, the PFS with gefitinib was better than chemotherapy at 12 months, while it was worse than chemotherapy at 3 months. However, when sub-group analysis was performed with regards to *EGFR* mutation status, it was clearly delineated that the use of gefitinib offered better PFS among patients with mutated *EGFR* (HR =0.48, P<0.001) and that the use of chemotherapy offered better PFS for patients with non-mutated *EGFR* (HR =2.85, P<0.001). Thus the IPASS study was revolutionary in that it demonstrated the benefits of gefitinib among *EGFR* mutated patients, while also demonstrating the non-effectiveness of gefitinib among those without *EGFR* mutations (5,36).

The phase-III OPTIMAL trial was initiated with the intention to compare the PFS benefit with erlotinib versus gemcitabine-carboplatin, when used as first line treatment in Chinese patients with *EGFR* mutated advanced NSCLC. The median PFS was better with erlotinib in comparison to chemotherapy (13.1 *vs.* 4.6 months, P<0.0001). Comparable results were also obtained from the European trial EURTAC which compared erlotinib versus chemotherapy for first line treatment of *EGFR* mutated European patients (37,38). The American Cancer Society has released a provisional clinical opinion that patients being considered for first-line therapy with an *EGFR*-TKI should be tested for *EGFR* mutations (39).

In addition to the fact the *EGFR*-TKIs offer better PFS in comparison to chemotherapy in *EGFR* mutated patients, the quality of life outcomes also were much better for patients treated with *EFGR*-TKI in comparison to chemotherapy. Obvious reasons include the ease of oral administration of TKIs, and the relative rarity of severe systemic side-effects with TKIs. The tolerability of *EGFR*-TKI have made it an agent feasible for addition to best supportive care among *EGFR* mutated adenocarcinoma patients with very poor performance status, who may otherwise be unfit for any other form of treatments. Occasionally dramatic regressions have been noticed, leading to a consequential improvement in performance status, offering a new lease of survival for a sub-population otherwise endowed with no hope (40).

### *Resistance to EGFR-TKI agents*

Resistance to TKI, can be of two sorts—intrinsic or acquired. Intrinsic resistance (also known as primary resistance) occurs among tumors which are inherently resistant to *EGFR*-TKIs. On the other hand, acquired resistance, also known as secondary resistance implies the development of non-response to *EGFR*-TKIs among patients who had initially responded to treatment (41). The causes of intrinsic and acquired resistance have been enlisted in *Table 1*.

 

**Table 1** Major causes of resistance to reversible oral EGFR-TKIs

| Primary resistance | Secondary resistance |
|---|---|
| *KRAS* mutation | T790 mutation |
| Mutations involving exon-20 | MET amplication |
| PTEN downregulation | HER3 activation |
| Other mutations mutually exclusive to EGFR | HGF overexpression |

KRAS, kirsten rat sarcoma oncogene; PTEN, phosphatase and tensin homolog; EGFR, epidermal growth factor receptor; MET, hepatocyte growth factor receptor; HER3, human epidermal receptor 3; HGF, hepatocyte growth factor.

Molecular causes for primary resistance include the presence of *KRAS* mutation, phosphatase and tensin homolog (*PTEN*) down-regulation, or the presence of exon-20 mutation. *KRAS* encodes a GTPase downstream of *EGFR*, and thus the activation of *KRAS* activates downstream signaling pathways independent of *EGFR* (42,43). Another cause for intrinsic resistance to *EGFR*-TKIs could be the down-regulation of *PTEN* expression. *PTEN* is an important negative regulator of the *PI3K* pathway which promotes proliferation and is anti-apoptotic. Thus, down-regulation of *PTEN* causes unhindered growth and survival (44). Patients with exon-20 mutations are known to show a lower response rate (of about 25%) to *EGFR*-TKIs in comparison to those patients with mutations affecting exons 19 or 21 (45,46). Additionally, a deletion polymorphism in the gene encoding the pro-apototic protein *BIM* (*BCL2-like 11*) is also likely to confer primary TKI resistance (47).

The issue of acquired (secondary) resistance is not new. The earliest identified phenomenon was the development of *BCR-ABL T315I* mutation which conferred resistance to imatinib (48). With regards to *EGFR* inhibitors, the most common cause (accounting for more half of cases) is the development of the *T790M* mutation, which occurs due to substitution of the amino-acid threonine at position 790 with methionine in exon-20. This leads to a lack of inhibitor specificity at the ATP binding site, leading to increased ATP affinity, the cause behind drug resistance (49-51). It is to be noted that the *T790M* mutation can occasionally be the cause of primary resistance too. Hepatocyte growth factor (HGF) receptor (*MET*) amplification reportedly accounts for about 20% of cases with secondary resistance (52). Other documented causes for secondary resistance include *HGF* over-expression and *HER3* activation (53,54).

The understanding regarding the possible development of secondary resistance among patients being treated with *EGFR*-TKI warrants thoughtful consideration towards the use of re-biopsy for secondary molecular assessments (46).

### *Management of resistance*

Gefinitib and erlotinib, both are reversible *EGFR* inhibitors. The use of irreversible dual *EGFR-HER2* inhibitors has been investigated for patients who develop secondary resistance to *EGFR-TKIs*. The LUX-1 study compared afatinib versus best supportive care in 585 patients whose disease progressed after one or two lines of chemotherapy and at least 12 weeks of erlotinib/gefitinib treatment. Though a PFS difference favored treatment with afatinib, there was no overall survival benefit. The overall response rate for afatinib was 11% (55). A phase-III trial (LUX-*lung 5*) has been initiated to assess the role of afatinib with weekly paclitaxel versus investigator's choice of single agent chemotherapy following afatinib monotherapy in patients failing treatment with gefitinib/erlotinib (56).

Dacomitinib is another irreversible pan-*HER* inhibitor which too has been tried among patients who have developed resistance to erlotinib/gefitinib. In a study involving 188 unselected patients of NSCLC who had failed two lines of prior chemotherapy, the use of dacomitinib was compared with erlotinib. Median PFS and objective response rates tended to be better with dacomitinib. Importantly, among *KRAS* mutated patients (primary resistance to gefitinib and erlotinib) the benefit with dacomitinib seemed to be much better (57,58).

### *EGFR testing: newer methods*

While direct sequencing DNA testing for mutations requires biopsy material, newer sequencing techniques have arrived, which offer ability to make cytology-based testing. Many of these PCR techniques have been already tested in clinical trials, such as in the Japanese trials NEJ 001 to 003 (59-61).

A recent study described the sensitivity and comparability among five commercially available next generation sequencing (NGS) techniques. All techniques were able to perform better than the older direct sequencing technique,

 

while being able to detect mutation types at >1% mutant deoxyribonucleic acid (DNA), with successful analysis rates of 91.4-100%. Thus given the remarkable sensitivity of NGS techniques, it can be stated that cytology derived DNA is a viable alternative to formalin fixed paraffin embedded tissues samples for analysis of mutations (29,62-64).

### *Significant of EGFR copy number*

In addition to testing the significance of *EGFR* mutation, the IPASS study also tested the significance of *EGFR* gene copy number. This sub-group analysis tended to imply that *EGFR* mutated patients with increased copy number tended to have better PFS increments with gefitinib in comparison to those without increased *EGFR* copy number (6,65).

### *KRAS mutations in lung adenocarcinoma*

*KRAS* mutations have been detected in about 30% of NSCLC, with the majority occurring in codons 12 and 13. The mutations result in constitutive activation of *RAS* signaling, which is present downstream of *EGFR* pathway. The presence of *KRAS* mutations have been the cause for a significant proportion of cases with primary resistance to *EGFR*-targeted therapy not only in lung cancer, but also in other malignancies such as colo-rectal and pancreatic cancers (41,66-70).

While *EGFR* mutations are more common in the young and in non-smokers, *KRAS* mutations tend to be noticed among the elderly and in heavy smokers (71). Though the quest to develop clinically effective *KRAS* targeted therapy has been mostly futile until this date, the importance of *KRAS* testing in lung adenocarcinomas lies in the fact that *KRAS* mutation positivity is an indicator of primary resistance to *EGFR*-TKIs.

*KRAS* mutation testing can be used as an alternative to *EGFR* testing, since *KRAS* and *EGFR* mutations are mostly mutually exclusive. The *KRAS* mutations are confined to only three codons, thus lowering the costs of mutation sequencing. Further, patients negative for both *EGFR* and *KRAS* mutations may harbor the chromosomal translocation *EML4-ALK*, which may be present in about 3-6% of patients (23,72,73).

### *ALK rearrangements in lung adenocarcinoma*

Many anaplastic lymphoma kinase (*ALK*) fusion oncogenes are known to occur as driver mutations. The most common among *ALK* fusion genes in lung adenocarcinoma is the echinoderm microtubule associated protein like 4 (*EML4*)-anaplastic lymphoma kinase (*ALK*) translocation fusion gene, which was first described as recently as in 2007 (74-76). The relative prevalence of *ALK* translocation mutations among lung adenocarcinoma patients is rather low (3-6%) in comparison to *EGFR* and *KRAS* mutations. However, if a population of adenocarcinoma patients were to be chosen as per a negative smoking history, and negative *EGFR/KRAS* mutation status, then the prevalence of *ALK* rearrangement mutations is significant (77).

Crizotinib is a TKI which is ATP-competitive for the receptor tyrosine kinases *ALK* and *MET* (78). Initial studies demonstrated an objective response rate of 56% and a median PFS of 10 months, which was impressive given the use in a population of patients pre-treated with prior chemotherapy regimens. This led to an accelerated approval for advanced *ALK* positive adenocarcinoma (79,80).

Although most patients with *ALK*-positive lung adenocarcinoma derive initial clinical benefit from crizotinib, the benefit is relatively short-lived because of the development of acquired resistance. The causes behind secondary resistance to crizotinib are a matter of ongoing research. As of now, only isolated cases have been studied with regards to the molecular basis behind secondary resistance. The currently reported mechanisms include *L1152R* and *L1196M* mutations. In vitro experiments have indicated that *EGFR* activation could be another mechanism behind crizotinib resistance (81,82).

Katayama *et al.* evaluated 18 patients with acquired resistance to crizotinib. A diverse variety of secondary mutations distributed throughtout the *ALK* tyrosine kinase domain region were noted in about 25% of the patients. Also, *ALK* fusion gene amplification could be behind secondary resistance (76).

### *MET amplication in lung adenocarcinoma*

*MET* receptor is a tyrosine kinase which can be activated by its ligand, *HGF*. Abnormal *MET* activity can be a result of various mechanisms such as *MET* gene amplification, *HGF* over-expression or *MET* gene mutation. While evidence implicating the effects of MET gene mutation in carcinogenesis is rather sparse, there is ample evidence that over-expression of *HGF*, or that *MET* gene amplification is associated with poor prognosis in lung adenocarcinoma (83-85).

Further, MET amplification is purported to be the cause of secondary resistance to *EGFR*-TKIs in up to 20% of patients with *EGFR* mutations. Thus, *MET* inhibition has

 

emerged as an important target since its inhibition can potentially restore sensitivity to *EGFR*-TKIs (86-88).

Various methods of targeting the *MET* pathway include the use of small molecule inhibitors of the *MET* receptor, or by the use of monoclonal antibodies against *HGF*. Small molecule inhibitors of *MET* are of two main categories—selective inhibitors such as tivantinib, and non-selective inhibitors such as crizotinib, foretinib, and cabozantinib. Various ongoing trials have been focusing not only upon *MET* targeting, but also upon dual-*MET-EGFR* blockade (89-91).

### *VEGF targeting in lung adenocarcinoma*

*VEGF* is an endothelial cell specific ligand which in important in regulating angiogenesis in normal and tumor tissues. VEGF-pathway is amenable to be targeted by three broad approaches: (I) via the use of monoclonal antibodies to target VEGF; (II) via the use of VEGF-Receptor inhibitors such as aflibercept; and (III) by the use of small molecular TKIs such as sunitinib and sorafenib to target the tyrosine kinase domain of VEGF-receptor (92-94).

The Eastern Cooperative Oncology Group (ECOG) protocol 4,599 and the European AVAIL were two large phase-III trials which were pivotal in the gaining of approval for bevacizumab in lung cancer (33,95,96). Histology dependence for the use of bevacizumab has been identified, with current indication for bevacizumab in lung cancer limited to 'non-squamous' histologies.

Though effective in certain populations, the use of bevacizumab is undeniably associated with significant treatment related morbidities and mortalities. Moreover, there have been no trials which have compared *EGFR*-TKIs versus bevacizumab, given that both agents seem to be used in a patient population with overlapping characteristics.

Aflibercept is a recombinant fusion protein used to target the VEGF, which has been investigated for use in platinum- and erlotinib- resistant pulmonary adenocarcinoma. Despite promise in early trials, the results in phase-III trials has been rather discouraging (97,98). Pazopanib, sunitinib, mosatenib and sorafenib are TKIs which act by inhibiting the *VEGFR*, *PDGFR* and the *RAS/RAF* pathways. These agents are yet to be proven for efficacy and safety in phase-III trials (99-102).

### *Other targets*

Unfortunately, the progress in respect of targeting the various other known oncogenic mutations in lung adenocarcinoma has rather been sparse. The development of targeted therapies against several other oncogenic mutations in pulmonary adenocarcinoma is an intense field of ongoing research with multiple preclinical & early trials attempting to develop agents to target mutations involving *BRAF*, *ROS1*, *RET*, *HER2*, *DDR2*, *FGFR1*, *ILGF-1* and various others (103-110).

## THE IASLC/ATS/ERS classification of lung adenocarcinoma

The past decade has witnessed the emergence of important understandings with regards to the management of lung cancer. The most important being the demonstration of 'histology dependence' for therapy with pemetrexed and bevacizumab, that these two agents are to be avoided in squamous cell carcinoma. The next most important finding was the discovery that activating mutations, such as *EGFR* and *ALK* are markers for response to specific TKIs. Further, many advances have occurred in the management of lung cancer, in terms of pathology, pulmonology, oncology and surgery. While the 2004 edition of the WHO classification of lung cancer was primarily a 'pathological classification', the need for a multidisciplinary classification was felt. The IALSC, the ATS and the ERS joined forces to set-up an international multidisciplinary panel consisting of pathologists, clinicians, surgeons, molecular biologists and radiologists. A systematic review of 11,368 citations, of which 312 articles were retrieved for full text review, was performed, and meetings were held to discuss the development of recommendations along with the new classification. All the recommendations were graded for their quality and strength by means of the grades of recommendation, assessment, development & evaluation (GRADE) approach. The new classification and the recommendations were published in the *Journal of Thoracic Oncology*, which is the official journal of the IASLC. An accompanying editorial was also published in the *European Respiratory Journal*, which is the official journal of the ERS (8,111).

This section of the review intends to provide a summary of the salient new recommendations in the 2011 IASLC/ATS/ERS lung adenocarcinoma classification. Readers are strongly recommended that they refer to the official publication of the classification published in the *Journal of Thoracic Oncology* for full details of the classification, the accompanying recommendations and the discussions supporting the recommendations (8).

### *Classification*

The conspicuous difference between the WHO-2004 and the IASLC/ATS/ERS-2011 classification is that the IASLC/ATS/ERS classification provides guidelines for classification based upon the nature of specimen. Up to about 70% of lung cancer patients present in advanced stages such that they are inoperable at diagnosis, these patients are generally treated with a non-surgical approach (chemotherapy, radiotherapy and molecular therapy) and resection specimens are hence unavailable. Underscoring this reality, the IASLC/ATS/ERS classification has provided standardized guidelines for pathological classification based on cytology or small biopsy samples (*Figure 1*). In addition, there are comprehensive guidelines for pathological classification of resection specimens too (*Table 2*). The other main difference from the WHO-2004 classification is that the IASLC/ATS/ERS classification has discarded the usage of the term 'bronchioloalveolar carcinoma' (BAC) since the tumors formerly classified as BAC can now fall under five distinct diagnoses (*Tables 3,4*). In addition to acinar, papillary and solid patterns, two new patterns, namely the 'micropapillary' and the 'lepidic' patterns have been added. The term 'mixed subtype adenocarcinoma' is not used in the IASLC/ATS/ERS classification.

While the WHO-2004 classification primarily was based upon the use of haematoxylin-eosin stains for pathological classification, the new IASLC/ATS/ERS classification attempts to integrate additional histopathological techniques such as mucin, thyroid transcription factor-1, and p-63 staining- all with an intention to be more capable of classifying NSCLC into adenocarcinoma and squamous cell carcinoma, given the implications upon treatment planning. Further, the IASLC/ATS/ERS classification recommends the use of *EGFR* mutation testing to further sub-classify adenocarcinoma patients so as to assess feasibility of treatment with *EGFR*-TKIs. A summary of new features in the IASLC/ATS/ERS classification over the WHO-2004 classifications are presented in the *Tables 3,4*.

### *Recommendations*

The use of histopathology forms the backbone of the new classification, even though efforts have been made in to integrate perspectives from clinical, radiological, molecular and surgical disciplines. In addition to providing classification guidelines, the new classification also provides a number of important evidence based recommendations.

One of the most important recommendations is the abandoning of the term 'BAC'. After publication of the 2004 edition of the WHO classification of lung cancer, the term BAC used to cause major confusions in interpretations, since it could be used for a rather wide range of entities-ranging from small solitary noninvasive peripheral lung tumors [which could have 100% 5-year disease specific survival (DSS)], to advanced invasive lesions with lepidic patterns (which could have low survival prospects) (112,113). Thus, the old term BAC has now been abandoned, and the spectrum of new nomenclatures which could fall into the old 'BAC' terminology is enlisted in *Table 4*.

Multiple studies had shown 100% DSS for patients with small solitary peripheral adenocarcinomas with pure lepidic growth (114-118). Similarly, 100% or near 100% 5-year DSS was observed for patients with minimally invasive tumors, if a cut off of 5 mm depth of invasion was used (119-123). Keeping these findings into consideration, the new classification has introduced two new concepts, namely 'adenocarcinoma *in situ*' (AIS) and 'minimally invasive adenocarcinoma' (MIA).

AIS refers to small (≤3 cm) localized adenocarcinoma with pure lepidic growth (growth restricted to neoplastic cells along preexisting alveolar structures), while lacking other patterns, and also marked by the absence of intraalveolar tumor cells, stromal, vascular, or pleural invasion. MIA refers to small (≤3 cm) solitary adenocarcinoma with a predominantly lepidic pattern and ≤5 mm maximum size of invasion. In case of multiple microinvasive areas in one tumor, the size of the largest invasive area, in the largest dimension should be measured. It must be noted that the MIA concept cannot be applied if the tumor contains foci of necrosis, or if there is any invasion of lymphatics, pleura or blood vessels.

Since it was noted that more than 90% of lung adenocarcinomas would fall into the WHO-2004 category of 'mixed subtype' adenocarcinoma, many independent groups assessed the feasibility of classifying lung adenocarcinomas as per the predominant histologic subtype. This methodology offers prospects of correlations between various histologic patterns with molecular features and clinical behavior (124-129). Thus, the term 'mixed-subtype adenocarcinoma' is not used in the IASLC/ATS/ERS-2011 classification. Semi-quantitative recording of patterns in 5% increments is adopted, and while the single most predominant pattern defines the diagnosis, it must be kept in mind that the percentages of the non-predominant patterns should also be recorded.

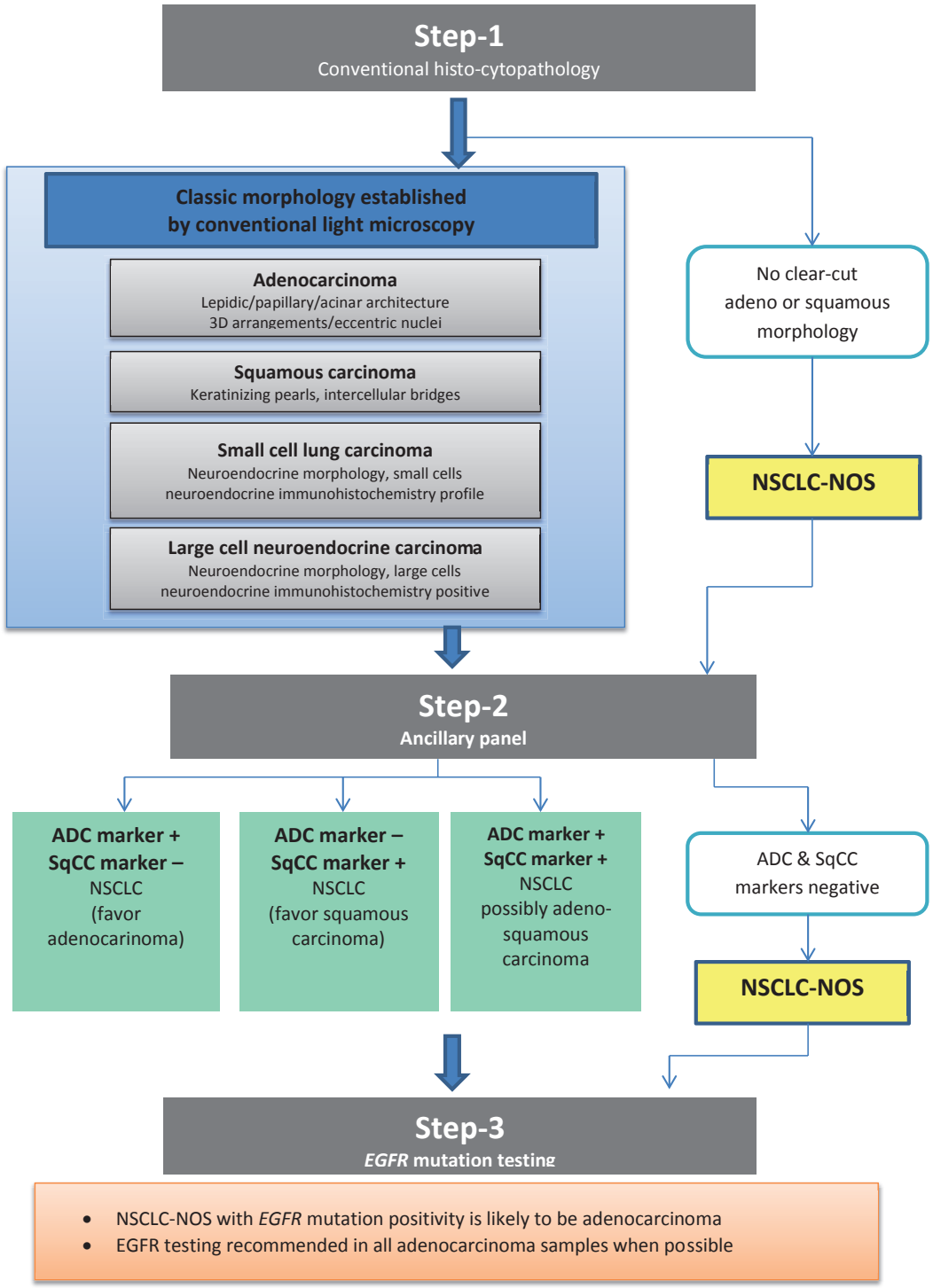


**Figure 1** Stepwise testing protocol for assessment of small biopsy/cytology samples. The first step involves conventional imaging using light microscopy with haematoxylin-eosin staining. In case of a non-small cell carcinoma 'not-otherwise specified' (NSCLC-NOS), the step-2 of the testing protocol suggests an ancillary panel with special stains and immunohistochemistry to help differentiate between squamous and adenocarcinomas. The step-3 involves EGFR mutation testing, which is recommended for all NSCLC-NOS since the presence of EGFR mutation most likely signifies adenocarcinoma histology. ADC, adenocarcinoma; SqCC, squamous cell carcinoma; EGFR, epidermal growth factor receptor.

 

**Table 2** IASLC/ATS/ERS—2011 classification of lung adenocarcinoma

| |
|---|
| Resection specimen based classification |
| Preinvasive lesions |
| Atypical adenomatous hyperplasia |
| Adenocarcinoma *in situ* |
| Nonmucinous |
| Mucinous |
| Mixed mucinous/nonmucinous |
| Minimally invasive adenocarcinoma |
| Nonmucinous |
| Mucinous |
| Mixed mucinous/nonmucinous |
| Invasive adenocarcinoma |
| Lepidic predominant |
| Acinar predominant |
| Papillary predominant |
| Micropapillary predominant |
| Solid predominant with mucin production |
| Variants of invasive adenocarcinoma |
| Invasive mucinous adenocarcinoma |
| Colloid |
| Fetal |
| Enteric |
| Biopsy/cytology specimen based classification |
| Morphologic adenocarcinoma patterns clearly present |
| Adenocarcinoma, describe identifiable patterns (lepidic, acinar, papillary, micropapillary, solid) |
| Adenocarcinoma with lepidic pattern (if pure lepidic pattern, to be mentioned that invasive component cannot be excluded on small biopsy/cytology specimen) |
| Mucinous adenocarcinoma (patterns to be described) |
| Adenocarcinoma with fetal pattern |
| Adenocarcinoma with colloid pattern |
| Adenocarcinoma with (describe patterns) and clear cell features |
| Adenocarcinoma with (describe patterns) and signet ring features |
| Morphologic adenocarcinoma patterns not present (possibility of adenocarcinoma suggested by ancillary stains) |
| 'Non-small cell lung carcinoma, favour adenocarcinoma' |
| Mention staining methodology used |

IASLC, International Association for the Study of Lung Cancer; ATS, American Thoracic Society; ERS, European Respiratory Society.

Though the WHO-2004 classification had mentioned the 'micropapillary pattern' of lung adenocarcinoma in the discussion section, there was no inclusion of this pattern as a formal subtype. Since tumors with a predominant micropapillary patterns are noted to be associated with poor prognosis, akin to adenocarcinomas with predominant solid pattern, the IASLC/ATS/ERS-2011 classification includes the 'micropapillary pattern' as a new major histological subtype (130-133). It is possible that even the presence of small amounts of micropapillary patterns may confer poor prognosis, and this is likely to be a focus of future research (134,135). It is noteworthy at this stage that a recent study described two types of micropapillary patterns that can occur in lung

 

**Table 3** Significant differences in the IASLC/ATS/ERS-2011 classification over the WHO-2004 classification

| Change | Description |
|---|---|
| Guidance provided for classification as per nature of pathologial specimen (resection specimens versus biopsy/cytology specimens) | Since 70% of lung cancer is unresectable at presentation, biopsy/cytology plays an important role in diagnosis and treatment guidance. The *IASLC/ATS/ERS* classification has provided classification guidelines for small biopsy/cytology samples, in addition to the classification for resection specimens |
| Recommendations on the use of special stains, immunohistochemistry and molecular testing | Given the paramount importance of classification of *NSCLC* into *ADC* and *SqCC*, recommendations are made so that in addition to routine haematoxyllin-eosin stains, optimal use of additional stains such as mucin, *p63* and *TTF-1* be done. Further, *EGFR* mutation testing too has been recommended for identifying eligibility for treatment with *EGFR-TKIs* |
| The term *BAC* is abandoned | The former term '*BAC*' can now be represented by five separate nomenclatures in the *IASLC/ATS/ERS* classification |
| New concepts of *AIS* and *MIA* | For use in resected specimens, new terminologies have been provided. Small peripheral adenocarcinomas with pure lepidic growth are classified as AIS. Small peripheral adenocarcinomas (≤3 cm) with predominantly lepidic growth with invasion of ≤5 mm is classified as *MIA*. *AIS* and *MIA* are recognized to have 100% or near 100% dispeace specific survival after complete resection |
| Recognition of new histological subtype: 'micropapillary' | Recognized to have a worse prognostic outcome, the micropapillary type has been added as a new sub-type of *ADC* |
| Omission of 'mixed subtype' variant, and importance placed upon predomiant histological pattern of invasive *ADC* for classification | Since >90% of all lung adenocarcinomas would fall into the 'mixed sub-type' as per the *WHO* 2004 classification, invasive adenocarcinomas are now classified upon their predominate pattern after comprehensive subtyping into either of lepidic, acinar, papillary, solid or micropapillary predominant subtypes. |
| Reclassification of former terminologies of mucinous cystadenocarcinoma, colloid *ADC*, fetal *ADC*, signet ring *ADC* and enteric *ADC* | Mucinous cystadenocarcinoma is now classified as 'invasive adenocarcinoma with enteric features'. The prior terminologies 'colloid adenocarcinoma and fetal carcinoma' are now renamed as 'invasive adenocarcinoma with colloid features', and 'invasive adenocarcinoma with fetal pattern', respectively. The prior terminologies of signet ring and clear cell carcinoma are now no more regarded as separate patterns. Instead, they are classified as 'adenocarcinoma with (describe patterns present) and signet ring features', and 'adenocarcinoma with (describe patterns present) and clear cell features', respectively |

ADC, adenocarcinoma; NSCLC, non-small cell lung carcinoma; SqCC, squamous cell carcinoma; TTF-1, thyroid transcription factor-1; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; BAC, bronchioloalveolar carcinoma; MIA, minimally invasive adenocarcinoma; AIS, adenocarcinoma *in situ*; WHO, World Health Organization; IASLC, International Association for the Study of Lung Cancer; ATS, American Thoracic Society; ERS, European Respiratory Society.

adenocarcinoma- namely the more common 'aerogenous micropapillary component' (AMPC) (with tumor cells floating in the alveolar spaces) and the 'stromal invasive micropapillary component' (SMPC) (with tumor cells found invading the stroma). Though the relative prevalence of SMPC (3.4%) was much smaller than the prevalance of AMPC (17.7%), it was observed on multivariate analysis that poorer DFS for stage I adenocarcinoma patients was associated with SMPC and not AMPC (136).

Rationale is also provided for the classification of invasive adenocarcinomas into mucinous and nonmucinous types. This is because that mucinous and nonmucinous adenocarcinomas seem to have major differences in terms of clinical behavior, pathology, and mutational status. While mucinous tumors are likely to show a strong correlation with *KRAS* mutations, non-mucinous tumors are more likely to display *EGFR* mutations (137-141).

While a summary of the recommendations is provided in *Table 5*, the authors again remind the readers to refer to the original manuscript published by Travis *et al.* in the *Journal of Thoracic Oncology* for full details and discussion (8).

 

**Table 4** Nomenclatures in the IASLC/ATS/ERS classification wherein the 'bronchioloalveolar carcioma' termiology was used in the WHO-2004 classification

| Entity | Characteristics | Comments |
|---|---|---|
| AIS | Pure lepidic growth, ≤3 cm, non-invasive | Usually non-mucinous, can rarely be mucinous. 100% DSS |
| MIA | Small ≤3 cm, predominantly lepidic, ≤5 mm invasion size | Usually non-mucinous, can rarely be mucinous. Near 100% DSS |
| LPA | Invasive ADC with lepidic growth as predominant component | 90% DSS at 5-years |
| Invasive non-mucinous ADC with lepidic component | Invasive nonmucinous ADC which can have small proportion of lepidic component in addition to predominantly having either of acinar, papillary, solid or micropapillary pattern | Prognosis variable. Depends upon co-existing patterns |
| Invasive mucinous ADC | Invasive mucinous ADC which has predominant component of lepidic growth | Prognosis variable |

AIS, adenocarcinoma *in situ*; MIA, minimally invasive adenocarcinoma; LPA, lepidic predominantly adenocarcinoma; ADC, adenocarcinoma; DSS, disease specific survival; IASLC, International Association for the Study of Lung Cancer; ATS, American Thoracic Society; ERS, European Respiratory Society.

**Table 5** Summary of important recommendations & considerations for good practise made in the IASLC/ATS/ERS-2011 classification

| |
|---|
| Pathology recommendations & considerations |
| The term 'NSCLC-NOS' to be used as sparingly as possible. Utilize staining with special stains/immunohistochemistry so as to favour adenocarcinoma versus squamous cell carcinoma |
| Important changes made over the WHO-2004 classification (mentioned in *Table 3*) |
| The terms AIS or MIA cannot be used in small biopsies or cytology specimens. |
| Tissue specimens should be appropriately managed so that in addition to diagnosis, molecular studies too can be performed |
| Cell blocks should be prepared from cytology samples, even with pleural fluids |
| The term 'non-squamous carcinoma' should not be used by pathologists, since it was a term used by clinicians to categorize patients eligible for treatment with pemetrexed and bevaciumab |
| Radiology recommendations & considerations |
| Radiologists performing biopsies/cytologies should obtain adequate tissues keeping in mind the needs for light microscopy, immunohistochemistry and molecular analysis |
| Utilize thin slice computed tomography in cases of 'part-solid' lesions so as to record the size of solid and ground glass components |
| Clinical recommendations & considerations |
| Given the prospects with tyrosine kinase inhibitors, patients with advanced lung ADC should be tested for presence of EGFR mutation |

NSCLC-NOS, non-small cell lung carcinoma 'not otherwise specified'; AIS, adenocarcinoma *in situ*; MIA, minimally invasive adenocarcinoma; EGFR, epidermal growth factor receptor; ADC, adenocarcinoma; WHO, World Health Organization; IASLC, International Association for the Study of Lung Cancer; ATS, American Thoracic Society; ERS, European Respiratory Society.

### *Research recommendations enlisted in the IASLC/ATS/ERS-2011 classification*

Members involved with the IASLC/ATS/ERS classification project had initiated research protocols in an attempt to develop data to test the IASLC/ATS/ERS guidelines. Studies conducted to validate the guidelines included projects on small biopsies, grading, molecular-histological correlations, AIS, MIA and major histological patterns (142-145). Though the classification guidelines were written backed by the best evidence available at the time of

 

drafting, there is a recognized need for further validation of the guidelines with more data. Indeed, the IASLC/ATS/ERS classification has also provided guidance on further research with regards to the classification and correlation with radiology, molecular biology and treatment of lung adenocarcinomas.

### *Pathology research prospects*

(I) Minimally invasive adenocarcinoma criteria—Since the MIA definition utilizes 5 mm as size cut-off for invasion size, the optimal method of measuring the size of the invasion component has to be established. Clarity also needs to be established as to the procedure with a tumor is associated with multiple foci of invasion.

(II) Impact of the nature of invasive component in MIA upon survival—It is to be established if the nature of the invasive component affects the disease free survival, since it is possible that micropapillary and solid patterns could possibly adversely affect survival.

(III) Impact of secondary patterns in invasive carcinoma—Since the guidelines use the most predominant pattern for sake of classification, it is to be investigated as to how the secondary patterns may affect prognosis. Given that micropapillary pattern is likely to adversely affect prognosis even when present in very small proportions of 1-5%, there is a possibility that more aggressive patterns may have a significant effect upon prognosis even if they are not the predominant pattern (146,147).

(IV) Ideal grading system—Since the current guidelines do not recommend any specific grading system, it may be necessary to investigate the utility of grading done by architectural grading versus nuclear grading.

(V) Patterns in metastatic sites—The prognostic implications of histological patterns in metastatic sites when compared to primary site is to be investigated.

(VI) Utility of frozen sections—The ability of pathologists to be able to distinguish in situ disease versus invasive disease upon examinations on frozen section is to be investigated.

(VII) Impact of ancillary diagnostic methods—The use of special stains with mucin, and with immunohistochemistry with TTF-1, p63 etc. have been suggested so as to reduce the frequency of NSCLC-NOS diagnoses. It is to be investigated as to how the classification by the use of special stains may possibly affect clinical outcomes, since existing data is based upon conventional light microscopy alone. Also, the utility of additional markers such as napsin-A is to be investigated.

### *Clinical and surgical research prospects*

(I) Adenocarcinoma in non-smokers—The clinical, epidemiological, molecular and histological characteristics of adenocarcinoma occurring in never-smokers is to be investigated.

(II) Impact of histological pattern in early stage lung cancer upon adjuvant therapy—It is to be investigated as to whether the histological patterns present affect the need for adjuvant chemotherapy in early stage lung cancer after resection. Given that the micropapillary pattern is predictive of a higher potential for metastases, patients of the same stage with aggressive histological features may require more intense treatment.

(III) Sub-lobar resections for some early adenocarcinomas—Though lobectomy is the standard surgical procedure for tumors less than 2 cm in size, it is to be investigated as to whether sub-lobar resections can offer equivalent survival for selected patients with radiologically detected ground-glass opacities which could be predictive of AIS and MIA.

(IV) Less morbid approaches for early stage lung adenocarcinoma—It is to be investigated to assess the results with thoractotomy versus video-assisted thoracic surgery (VATS) with regards to morbidity and survival outcomes.

(V) Can lymph-nodal dissection be omitted in some early stage adenocarinomas? Recent data suggests that some very early stage adenocarcinomas (especially the ground glass opacity lesions diagnosed radiologically) may not require lymph-nodal dissection (148).

(VI) Protocol for multi-focal adenocarcinomas—There is need to evolve a treatment algorithm for management of multiple lesions with regards to nature, number, size, locations, synchronous versus metachronous lesions, and primary versus metastatic lesions.

### *Molecular research prospects*

(I) Histology-molecular correlations to be assessed-While certain histologies are likely to be associated with certain mutations (e.g., Signet ring cell

 

histology with *EML4-ALK* mutations, mucinous adenocarcinoma with *KRAS*, non-mucinous adenocarinoma with *EGFR*), further research is required to assess the frequency and strength of these associations (137-141).

(II) Micro-RNA, genomics, and proteomics—evaluation is required to assess if micro-RNA, genomics and proteomics testing can provide information regarding risk stratification and outcome prediction in lung adenocarinomas.

### *Radiology research prospects*

(I) Radiological measurement of tumor—The ideal method of measurement is to be established. With regards to part-solid and part-ground glass lesions, the importance of the relative proportions of the solid and 'ground glass components' is to be established.

(II) Possibility of use of computed tomography (CT) attenuation data analysis to differentiate between multiple-primaries versus metastases to be investigated

(III) Attempts to establish molecular correlations with radiological findings such as ground glass opacities and standardized uptake value (SUV) on positron emission tomography to be embarked upon.

(IV) Impact of the new classification upon CT-based screening for lung cancer to be evaluated.

### *Potential implications of the IASLC/ATS/ERS classification upon staging*

The recommendations within the IASLC/ATS/ERS classification has potential implications upon future editions of the tumor-nodes-metastases (TNM) staging for lung cancer. First of all, it may be feasible to provide separate staging systems for lung cancer as per histology, similar to the current version of staging for esophageal carcinoma which has separate staging systems for squamous and adenocarcinomas. Secondly, it may be possible to integrate the new concepts of AIS and MIA into TNM staging systems by away similar to the current staging system for breast cancer, by incorporation of the terms Tis (for *in-situ* lesions) and Tmi (for microinvasive lesions). It is also to be seen if radiological assessments of tumor volume with regards to the invasive and the non-invasive components by volumetric measurements of the solid and ground-glass components could be used in staging for patients who may be treated by non-surgical approaches, such as with radical stereotactic ablative radiotherapy (SABR). However, much data needs to be accumulated before the drafting of the next edition of the TNM-AJCC staging for any changes to be incorporated (149-151).

### *Potential controversial issues with the IASLC/ATS/ERS classification*

It is to be noted that the IASLC/ATS/ERS classification in itself acknowledges weaknesses and also has provided guidelines for further research to strengthen or refute the recommendations provided within the classification system. However, there are some issues which confer a degree of ambiguity on all possible interpretations on classification made using the IASLC/ATS/ERS guidelines.

Given the complexity introduced into the histological sub-typing, it is likely that reproducibility with regards to inter-observer variations among pathologists will be a rather serious issue. The most likely areas of confusion will be with regards to assigning a diagnosis of lepidic versus acinar pattern, and in differentiating the micropapillary and the papillary patterns (152,153).

Given that the IASLC/ATS/ERS classification has deleted the 'mixed-subtype' category in favor of typing by the most-predominant histological pattern, important questions remain to be answered. Given that solid and micropapillary subtypes are considered to be of poor prognostic significance, it is likely that the presence of these patterns even in small proportions could adversely affect prognosis. As in the case with the use of the Gleason score in prostate cancer, it is to be investigated if there is a rationale in providing weightage to non-predominant patterns. However, the IASLC/ATS/ERS does encourage research on the issue of non-predominant patterns as part of its pathology research recommendations.

Other questions to be answered include the relative importance of architectural grading in comparison to nuclear grading, the impact of inflammation or stromal desmoplasia upon determination of invasion size, and the best possible methodology for quantifying depth/size of invasion.

## Future prospects in lung adenocarcinoma

### *MicroRNAs (miRNA)—potential in lung cancer diagnosis, prognostification and treatment*

miRNA are non-coding RNA which act as post-transciptional regulators of gene expression (154). Importance in research

on miRNAs is justified since miRNAs are known to be involved in the ability to regulate multiple genetic pathways, having both pro- and anti-oncogenic abilities (155). miRNAs are thought to have great potential in terms of diagnosis, prognostification and treatment of lung cancers.

The use of miRNA detection algorithms for lung cancer diagnosis is an attractive concept since it allows methods of non-invasive lung cancer diagnosis using blood miRNA expression. miRNA-25 and 223 were identified as biomarkers of NSCLC, and then the detection of a pattern characterized by overexpression of miRNAs-155, 182 and 197 could be used to separate patients with lung cancer from cancer free subjects. miRNA based diagnosis has also raised possibility of the use of sputum for the purpose of lung cancer diagnosis (156). Various *i* signatures can also possibly used to differentiate between adenocarcinoma and squamous cell carcinomas. Recent studies have demonstrated the effectiveness of using miRNA-205 as a marker for squamous cell lung cancer. Further, it is likely that miRNA signatures will help in the differentiation of primary lung tumors from lung metastases. Also, various studies have enumerated miRNA signatures which could possibly help in the prognostification of lung cancer patients. Much research however needs to be put in efforts of standardizing the potential protocols for the use of miRNAs in diagnosis and prognostification in routine clinical use. Since miRNAs are known to be involved in pro- and anit-oncogenic effects, efforts are underway to develop clinically feasible techniques to utilize miRNA based treatments for the treatment of lung cancer. Potential approaches include the inhibition of oncogenic miRNAs, as well as in enhancing the functions of tumor suppressive miRNAs (157-160).

### *Cancer stem cell (CSC) research*

Treatment with chemotherapy, radiotherapy or molecular targeted therapy often downsizes the tumor, many times to even cause clinical complete remissions. However, a significant majority of these tumors ultimately recur—either locally or at distant sites. The CSC hypothesis explains this phenomenon. Given that the bulk of the tumor can be eradicated by cytotoxic agents, it is possible that cancer stem cells remain quiescent, hence conferring themselves protection against cytotoxic agents and ionizing radiation-which basically are active against dividing cells. These surviving CSCs reactivate later on, leading to relapse (161).

Regulation of CSCs is likely mediated by the Hedgehog, Wnt and the Notch signaling pathways (162). Eradication of CSCs would lead to prospects of 'cure' after complete remissions after standard therapies have been achieved. Efforts are on to develop methods of detecting CSC related biomarkers for clinical use (163-165). With regards to targeting of CSC associated pathways, clinical progress has been steady- with ongoing experiments using cyclopamine to target the hedgehog pathway; and with promising potential with the use of gamma-secretase inhibitors to target the notch pathways (166,167). Also, it is postulated that CSCs can be eradicated by the use of very high doses of radiotherapy, as would occur with the use of SABR in eligible patients (168).

### *Potential with SABR*

Stereotactic-irradiation combines highly conformal delivery of radiation to selected volumes at large doses per fraction, with the treatment completed typically within one to five fractions. The radiobiological equivalence of doses delivered by stereotactic-irradiation (often beyond 80-100 Gy) is much higher in comparison to the doses achievable by conventional fractionation. The recent emergence of various technological innovations with regards to image guidance and precision radiotherapy has allowed the routine use of hypofractionated stereotactic radiotherapy in almost any site in the body. At the high fraction sizes used in stereotactic-irradiation, evidence suggests the role of various radiobiological mechanisms of actions, which are not traditionally associated with conventional radiotherapy. These include induction of endothelial damage, cell membrane damage, organelle damage, ceramide pathway activation and eradication of dormant tumor cells (168-171).

SABR is also referred to by other acronyms such as stereotactic body radiotherapy (SBRT), extracranial sterotactic RT (ESRT) and fractionated sterotactic radiotherapy (FSRT). Initial studies of SABR in lung cancer involved early stage NSCLC among patients who were inoperable due to medical reasons. It was noted that the approach was comparable to surgery in terms of results, while being very tolerable. Further, the use of SABR is not associated with a significant risk of immediate mortality unlike with thoracic surgery. Also, the local control rates are excellent, consistently exceeding 95% at 1-year, and median overall-survival consistently exceeds 5-year. SABR is now being tested by a number of trials for operable lung cancers too. An outline of the recent trials addressing the use of SABR in early stage NSCLC is provided in *Table 6* (172-177).

**Table 6** Trials using SABR for early stage lung cancers

| Trial | Ref | Remarks | Outcome |
|---|---|---|---|
| Lagerwaard *et al.*; 2012 | (172) | SABR (60 Gy in 3, 5 or 8 Fc depending on tumour size and location) in potentially operable patients | LC at 1 and 3 years were 98% and 93% respectively. Less than 3% risk of grade 3 toxicity. Median OS exceeded 5 years. 30-day post-procedure related mortality was 0% |
| Chan *et al.*; 2012 | (173) | 16 stage-1 NSCLC medically inoperable with a median age of 82 years | 2-year LC, DFS and OS were 91%, 71% and 87% respectively |
| Haasbeek *et al.*; 2011 | (174) | SABR (at 7.5 Gy × 8 Fc) for centrally located early NSCLC | Distant metastases and comorbidities were the predominant cause of death. 3-year LC and OS rates were 90.2% and 51.1 respectively |
| Fakiris *et al.*; 2009 | (175) | Phase-II prospective trial involving 70 early staged NSCLC treated with SABR (60-66 Gy) | SABR results in high rates of local control in medically inoperable patients of early NSCLC. Cancer specific survival at 3 years was 81.7% |
| Timmerman *et al.*; 2010 | (176) | Phase-II prospective trial of Early stage NSCLC who were medically inoperable treated with SABR (18 Gy × 3 Fc) | 3-year DFS and OS were 48.3% and 55.8% respectively |

LC, local contol; NSCLC, non small cell lung carcinoma; DFS, disease free survival; OS, overall survival; Fc, fraction; SABR, stereotactic ablative radiotherapy.

It must be emphasized here that SABR at present can only be applied to small target volumes as seen in stage-I and II tumours, for patients who are inoperable for any reasons. Also, given that the use of SABR will cause destruction of the tumor, all required pathological testing should be established by means of small biopsy/cytology testing prior to the initiation of SABR.

### *Emerging prognostic and predictive indicators*

Various histopathological prognostic factors for lung adenocarcinoma have already been well described in existing literature. Factors such as intratumoral vessel invasion, visceral pleural invasion, perineural invasion, mitotic index, Ki-67 labelling index, histological grade, presence of tumor necrosis etc. have been established as being prognostic for tumor recurrence after complete resection (178-183).

Advances in nuclear medicine have also led to the emergence of new prognostic indicators such as with a high SUV in a FDG-PET scan being indicative of poor differentiation and increased propensity for lymph-nodal involvement.

The advances in genetic profiling has led to the emergence of prognostic molecular assays for various malignancies- such as with the OncotypeDX (TM) and MammaPrint (TM) for breast cancer—which are designed to be predictive of relapse after surgery for early breast cancer, and to help assist in deciding the necessity for adjuvant chemotherapy. Similar molecular profiling systems are also available for colon cancer. Though prognostic molecular assays for lung adenocarcinoma is not yet commercially available, the feasibility for the same was described recently by Kratz *et al.*, who utilized a 14-gene expression assay to predict patient at high risk of mortality after surgical resection (184-187). If validated in large trials, it is likely that personalized decisions regarding the use adjuvant chemotherapy after surgical resection of early stage lung adenocarcinoma can be feasible.

In addition to prognostic factors, recent years have seen the emergence of 'predictive factors'. Which though not indicative of patient prognosis are useful in being able to 'predict' responsiveness to specific agents. The excision repair cross-complementing group 1 (*ERCC1*) and the ribonucleotide reductase M1 (*RRM1*) genes are naturally involved in repair of *DNA*, and overexpression of these genes are thought to reduce efficacy of platinum compounds and gemcitabine, respectively. Also, advances in molecular profiling have led to the prediction of sensitivity to, or resistance for specific molecular targeting agents (188-190).

## Conclusions

There has been a deluge of new discoveries in relation to the management of lung adenocarcinomas. The realization

 

of the importance of sub-classifying NSCLC into more specific histologies was first felt due to the availability of histology suited therapies such as pemetrexed and bevacizumab. Then, the availability of effective molecular targeted therapies for specific mutations has now made it important for inculcating molecular testing as part of investigation protocols for lung adenocarcinomas. The recent IASLC/ATS/ERS classification of lung adenocarcinoma is a step forward, in that it has made efforts towards inclusion of perspectives not only from the advances in pathology, but also from those in molecular biology, radiology, oncology and surgery. The IASLC/ATS/ERS classification comes with major procedural amendments over the WHO-2004 for the classification of lung adenocarcinomas. The IASLC/ATS/ERS has also provided important guidelines for research, so as to help gain evidence towards strengthening the recommendations put forth in the classification. In the coming years, it is possible that accelerated developments in the field of molecular biology, genetic profiling, radiology, nuclear medicine, surgery, radiotherapy and chemotherapy will bring forth major changes in the way lung adenocarcinoma is diagnosed and treated.

## Acknowledgements

*Disclosure:* The authors declare no conflict of interest.

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**Cite this article as:** Revannasiddaiah S, Thakur P, Bhardwaj B, Susheela SP, Madabhavi I. Pulmonary adenocarcinoma: implications of the recent advances in molecular biology, treatment and the IASLC/ATS/ERS classification. J Thorac Dis 2014;6(S5):S502-S525. doi: 10.3978/j.issn.2072-1439.2014.05.19

 