Exploring histopathological and serum biomarkers in lung adenocarcinoma: Clinical applications and translational opportunities (Review)

MIGUEL A. ORTEGA[1-3], FÁTIMA NAVARRO[1,2,4], LEONEL PEKAREK[1,2,5], OSCAR FRAILE-MARTÍNEZ[1,2], CIELO GARCÍA-MONTERO[1,2], MIGUEL A. SAEZ[1,2,6], MONICA ARROYO[1,2,4], JORGE MONSERRAT[1,2] and MELCHOR ALVAREZ-MON[1,2,7]

[1]Department of Medicine and Medical Specialties, Faculty of Medicine and Health Sciences, University of Alcala, Alcala de Henares, 28801 Madrid; [2]Ramon and Cajal Institute of Sanitary Research, 28034 Madrid; [3]Cancer Registry and Pathology Department, Prince of Asturias University Hospital; [4]Oncology Service, Prince of Asturias University Hospital, Alcala de Henares, 28806 Madrid; [5]Oncology Service, Guadalajara University Hospital, 19002 Guadalajara; [6]Pathological Anatomy Service, Central University Hospital of Defence-UAH Madrid, Alcala de Henares, 28801 Madrid; [7]Immune System Diseases-Rheumatology, Oncology Service an Internal Medicine, University Hospital Príncipe de Asturias, Alcala de Henares, 28806 Madrid, Spain

Abstract. Lung cancer represents one of the most common neoplasms and the main cause of cancer-associated death worldwide. Its relationship with different risk factors such as tobacco, which is its main etiological factor, has been clearly established and despite the numerous advances achieved in the diagnosis, treatment and follow-up of these patients, the life expectancy of these patients is notably limited. Furthermore, its treatment is not exempt from comorbidities and frequently it neither provides optimal control of the disease nor improve the quality of life of these patients. Despite the possibility of performing screening tests in patients at risk, their implementation in daily clinical practice is complex and most of them are diagnosed at an advanced stage of their disease where systemic radiotherapy or chemotherapy treatments slightly improve their prognosis. Lung adenocarcinoma is the most representative type of lung cancer, with specific epidemiological, molecular and clinical features. Thus, a growing number of studies are being conducted to find potential therapeutic targets based on the study of different molecular pathways, improving the outcome for these patients. In addition, a broad spectrum of serological, immunohistochemical and genetic markers are being evaluated for use in the screening and follow-up of these patients in daily clinical practice, but unlike for other tumors, they are currently not implemented in the early diagnosis of the disease. Therefore, the aim of the present review was to summarize the main advances that have occurred in the development of serological and histological markers and their therapeutic implications in patients diagnosed with lung adenocarcinoma, explaining the limitations that have been observed and analyzing the future perspectives in the clinical management of this disease.

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Correspondence to: Professor Miguel A. Ortega, Department of Medicine and Medical Specialties, Faculty of Medicine and Health Sciences, University of Alcalá, Alcalá de Henares, Ctra, Madrid-Barcelona km 33.6, 28801 Madrid, Spain
E-mail: miguelangel.ortega@edu.uah.es

1. Introduction

Lung cancer is the second most common neoplasm in the world, but the first in males, and the leading cause of cancer-associated death. In 2020, there were >2.2 million new cases globally, causing >1.8 million deaths (1). Despite the remarkable advances made in the diagnosis, immunotherapy and monitoring of the disease, the average 5-year survival rate is ~23-27%. Lung cancer is characterized as having a high lethality and comorbidity, and the majority of patients are diagnosed in advanced stages where curative surgical options are limited (2). It should also be noted

that mortality data are similar in developed and emerging countries. Even for patients who underwent curative surgery for the clinical management of localized tumors, the 5-year survival rate is only 65%, demonstrating the aggressiveness of these tumors despite those patients having been diagnosed in early stages. In addition, >70% of patients diagnosed with lung cancer have locoregional or metastatic lymphatic spread, decreasing the probability of survival at 5 years to ~33 and ~7%, respectively (3). In developed countries, the incidence of lung cancer has decreased in recent years thanks to measures to prevent tobacco use and control occupational exposure to asbestos, since the former represents the main risk factor in the general population. These primary prevention measures have caused a 45% decrease in lung cancer mortality in males in the 1990-2015 period and a 19% decrease in females in the 2002-2015 period (4). Other risk factors for lung cancer have been described and include a multitude of agents such as radon, arsenic, benzopyrenes, asbestos, infections such as tuberculosis, and environmental pollution, although their contribution to global cases is only minor, as tobacco smoking is responsible for up to 90% of lung cancer cases (5). This is due to the fact that combustion of tobacco causes the release of polycyclic hydrocarbons, nitrosamines, nitrates and 60 other carcinogens, which induce alterations in DNA repair mechanisms, cell cycle control and dysplasia processes that lead to histological degeneration, followed by predominating proliferation and invasion of aberrant malignant cells (6). Smoking cessation is associated with a clear decrease in the relative risk of developing lung cancer after 10-15 years (7). It should be noted that ~1.1 billion individuals smoke in the world and of these, 10-20% of smokers may develop lung cancer (8). On the other hand, lung cancer is more frequent in males and the maximum incidence by age is between 80 and 90 years (9).

The histological varieties of lung cancer have been traditionally classified by prognostic, pathological and therapeutic factors. They have been differentiated into small cell lung cancer (SCLC) characterized by small cells with a very poor prognosis and mostly associated with paraneoplastic syndromes (Cushing's syndrome, Syndrome of inappropriate antidiuretic hormone secretion) and non-SCLC (NSCLC), which are subdivided into squamous cell carcinoma, large cell carcinoma and lung adenocarcinoma (10). The subtype most associated with tobacco exposure is squamous cell cancer, but the most frequent subtype is lung adenocarcinoma, which accounts for >60% of non-small cell tumors (11). Lung adenocarcinoma has unique histological, radiological, epidemiological, molecular and clinical characteristics compared with other tumors. For instance, the incidence of lung adenocarcinoma is similar in males and females, and it is the lung neoplasm with the highest incidence in individuals who have never smoked and <45 years of age (12). Histologically, lung adenocarcinoma is composed of bronchial glands with a tendency to papillary configuration that degenerate due to deterioration of type II pneumocytes, generating atypical alveolar hyperplasia and later a truly invasive neoplasia. At the pathological level, the 2021 World Health Organization (WHO) classification allows lesions to be differentiated according to their invasive potential, classifying them into minimally invasive mucinous or non-mucinous lesions, and invasive non-mucinous adenocarcinoma, which in turn may be subclassified in acinar, papillary, micropapillary

and solid tumors. Other less frequent types include invasive mixed mucinous lesions, colloid adenocarcinoma, fetal or enteric type, each of them with diagnostic, prognostic and clinical peculiarities (13).

Regarding the early diagnosis of this disease, it should be noted that there are screening programs for smokers that have been evaluated by low-dose computed tomography (CT) of the chest as approved by the US Preventive Task Force, but in clinical practice, they are difficult to apply, which means that most patients are diagnosed in advanced stages of the disease (14). With respect to the clinical management of pulmonary nodules, evaluation with CT, thoracoscopy, mediastinoscopy and positron emission tomography-CT have allowed to improve the diagnosis in the initial stages in these patients, which still represent a small proportion of them and it is associated with a complex management, subjecting the patients to a great level of emotional stress with a follow-up that may last several months (15). Unlike other tumors, such as pancreatic adenocarcinoma, ovarian, breast and testicular neoplasms, where markers such as CA19-9, CA125, CA15.3 or carcinoembryonic antigen (CEA) may be used, there are currently no serological markers in daily clinical practice that may help diagnose lung cancer (16). Of note, most patients initially present with constitutional syndrome, hemoptysis or cough. It is also common for numerous patients to present with superior vena cava syndrome, Horner's syndrome, compression of the brachial nerve or pericardial effusion (17). Although it is true that SCLC is the lung neoplasm that has been most clearly associated with paraneoplastic syndromes, patients with lung adenocarcinoma may present with acanthosis nigricans, dermatomyositis or and Trousseau syndrome (18).

The therapy of these tumors is different according to the time-point of diagnosis. In initial stages (I, II and IIIA), where the tumor is susceptible to surgical treatment, patients may undergo radiotherapy, neoadjuvant chemotherapy and subsequent surgery if they are surgical candidates. In more advanced stages, such as IIIB and IV, where there is mediastinal or subcarinal involvement, contralateral pulmonary invasion or metastatic spread, they are treated with chemoradiotherapy. Furthermore, it is possible to perform a histopathological study in order to administer specific immunotherapy regimes (19,20). In this sense, the use of immunotherapy has been a real advance in recent years as it has improved the prognosis in patients with disseminated disease, but even so, >80% of patients diagnosed with lung adenocarcinoma in the advanced stage do not survive for >5 years (21). Similarly, the lack of early diagnosis or serological markers is a real challenge in the early diagnosis of this disease. Hence, the purpose of the present review was to discuss the main immunohistochemical and diagnostic markers that not only help in the initial staging but may also be useful in the follow-up of patients in both advanced and early stages. Furthermore, the state-of-the-art of potential serological markers used in these patients was equally revisited, including promising approaches such as circulating tumor cells (CTCs), microRNAs (miRNAs/miRs) and exosomes.

2. Molecular and histological markers in lung cancer

In recent years, the histopathological classification of lung cancer has been modified by the discovery of novel histological

markers, targeted therapies and numerous molecular markers, which have completely changed the diagnosis and prognosis of these patients. Since 2015, the WHO molecular markers have guided the treatment to be followed based on the expression of markers with a last update in 2021, taking even more into account the relationship of molecular markers with the diagnosis of lung adenocarcinoma (22). Classically, the treatment of metastatic lung adenocarcinoma has been based on the use of systemic chemotherapy regimens not only to limit the extension and limit tumor progression, but also for palliative purposes to reduce tumor burden and improve the symptoms of patients in the final stages of the disease. In the last 20 years, discoveries in different molecular pathways have provided a better understanding of the underlying pathophysiology of the mechanisms of carcinogenesis, vascular invasion, proliferation and metastatic capacity. These mechanisms range from epigenetic and genetic markers to cytoplasmic receptors and metabolic pathways, which has led to the development of molecular targets in selected patients (23). Despite their efficacy, chemotherapy regimens have multiple common adverse effects that may even be lethal for numerous patients, so the use of immunotherapy not only reduces the risk of adverse effects, but its administration is also associated with better long-term results (24). Of note, prior to the application of directed therapies, the expression of different biomarkers must be analyzed to establish which patients may be candidates for these therapies. Among the numerous biomarkers available, the most notable are somatic genetic alterations called driver mutations. Driver mutations are genetic alterations that occur in the preneoplastic phase of tumor cells and that confer mitotic and invasive activity (25). Likewise, there are so-called passenger mutations that occur in tumor cells but with limited action in the invasive neoplastic process and that may be observed in non-tumor cells (26).

The metabolic pathways that confer survival to tumor cells in these patients derived from the expression of driver mutations have been studied in recent years. The identification of driver mutations has been and remains to be a central subject of study. For instance, Kris et al (27) examined 1,007 patients with lung adenocarcinoma and found driver mutations in 64% of them, and genetic alterations in KRAS, EGFR and anaplastic lymphoma kinase (ALK) were frequent. Of the 1,007 patients, 28% were candidates for receiving targeted therapy, achieving a median survival of 3.5 years compared to 2.4 years in those who did not receive targeted therapy (27). Currently, the development of new drugs and novel formulations of existing ones allow for expanding the number of candidates, which has undoubtedly made it possible to improve average survival. That is why in recent years, associations such as the WHO, College of American Pathologists, Spanish Society of Medical Oncology and Spanish Society of Pathology, among others, recommend genotyping for EGFR (Epidermal growth factor receptor) and BRAF (V-Raf murine sarcoma viral oncogene homolog B) V600E mutations, ALK (anaplastic lymphoma kinase) and c-ros oncogene 1 (ROS1) rearrangement and, in the case of non-smokers, light smokers or people <50 years of age, request the examination of programmed death ligand 1 (PDL1) expression (28-30). Genotyping may be obtained by different techniques, such as DNA sequencing, next-generation sequencing (NGS), DNA

allele-specific testing, immunohistochemistry or in situ fluorescence (31). It should be noted that NGS has provided a true progression in the analysis of mutations in lung cancer, since it allows the analysis of a wide variety of genes whose information may be stored and later studied to analyze the relevance of undescribed mutations, which makes it possible to have a mutation database (32). In addition, the efficacy of studying driver mutations in CTCs in peripheral blood has been noted, although with a sensitivity limited to 60-80%, which may restrict the possibility it of being applicable in targeted treatments in addition to not allowing the measurement of PDL1 under certain conditions (33). For instance, the mutation of EGFR, present in ~15% of adenocarcinomas in western countries, determines the activation of metabolic pathways such as RAS, PI3K or phospholipase C that cause an increase in cell survival and tumoral growth (34). The EGFR mutation is subsidiary to treatment with tyrosine kinase inhibitors, such as erlotinib, afatinib or osimertinib. Of note, in Asian populations, the presence of EGFR mutations has been described in >50% of adenocarcinomas (35). It should also be highlighted that there are mutations within the EGFR gene in exons 18-21, being the most frequent those occurred in exons 19 and 21, each with different prognostic implications (36). In this sense, Yoon et al (37) analyzed 1,020 patients with lung adenocarcinoma and obtained a positive result for EGFR in 388 patients (~38%). Of the 388 patients, 51% had a mutation in exon 19 with a median survival of 29.9 months, whereas for those who had a mutation in exon 21, the median survival was 20.6 months. In a meta-analysis conducted by Zhang et al (38) that included 13 different studies, exon 19 deletion appeared to be associated with longer progression-free survival compared to L858 mutation at exon 21 after treatment with first-line EGFR-tyrosine kinase inhibitors (EGFR-TKIs). Likewise, patients with the T790M mutation of exon 20, where a threonine is replaced by a methionine in position 790, may be resistant to first- and second-generation EGFR-TKI, although this resistance does not occur with third-generation agents, such as osimertinib, which has been evaluated in clinical trials such as AURA 3 (39,40).

The rearrangement of ALK has also been described, which has an incidence of ~4% in patients with lung adenocarcinoma (41). ALK rearrangement leads to alterations in the signaling of a type of insulin-related receptor tyrosine kinase present in neurons of the central nervous system that is frequently altered in anaplastic long cell lymphoma, inflammatory myofibroblastic tumor and neuroblastoma. All of this determines the activation of the echinoderm microtubule-associated-protein-like 4-ALK complex, which causes alterations in the correct formation of microtubules and thus proliferation and migration of tumor cells (42). It should be noted that ALK rearrangement has special clinical features. For instance, patients with ALK rearrangement are more likely to develop brain metastases (43). In addition, these patients tend to be younger, with a mean age of 52 years, and non-smokers (44). It should be noted that cases with ALK rearrangement respond to treatments with crizotinib, ceritinib or lorlatinib, among others, determining average survival times of up to 48 months in advanced stages (45).

On the other hand, cases with abnormalities of mesenchymal epithelial transition factor receptor (MET) tyrosine

kinase in exon 14-skipping mutations and amplifications present in up to 6% of lung adenocarcinomas-responded to therapies with capmatinib, tepotinib and crizotinib, which made it possible to control the progression of adenocarcinoma in these patients (46). MET is activated by a single ligand known as hepatocyte growth factor, driving the activation of pathways such as AKT, ERK/MAPK or STAT3, promoting an increase in cell survival, proliferation and migration (47).

Likewise, the rearrangement of the protooncogene RET has been described, which determines an activation of cytosolic kinases derived from RET, which occurs in 1-2% of patients with lung adenocarcinoma (48). RET rearrangement is mainly found in young patients without a history of smoking and, like EFGFR or ALK mutations, it is associated with a high probability of metastatic progression in the brain (49). RET rearrangement is amenable to treatment with selpercatinib and pralsetinib therapy as demonstrated in the LIBRETTO-001 and ARROW clinical trials with response rates of 64% maintained for ~18 months (50,51).

Mutations in BRAF and the subsequent activation of the MAPK pathway are present in ~4% of patients and are usually associated with non-smoking patients (52). Given the favorable responses shown in patients with other aggressive neoplasms such as melanoma, the use of different targeted therapies has been studied, with the V600E mutation being the most frequent in lung adenocarcinoma. Patients with BRAF mutations tend to have a better prognosis than those without mutations, as this conditions a better response to immunotherapeutic treatment with dabrafenib and trametinib (53). Another type of molecular alteration described are alterations in tropomyosin receptor kinases, which are present in <1% of adenocarcinomas that are candidates for treatment with larotrectinib and entrectinib, reaching response rates of up to 80% and a median survival of 90 months (54).

Rearrangement of ROS1, which is present in up to 2% of lung adenocarcinomas, is also worth mentioning (55). This alteration, similar to the others, is much more frequent in patients with adenocarcinoma, being more common in young patients and non-smokers. This mutation usually occurs between the ROS1 and CD74 oncogene and is accompanied by an activation of metabolic pathways such as JAK/STAT, PI3K/AKT and MAPK/ERK, causing an increase in cell proliferation, cell survival and histological invasion capacity (56). One of the therapies that has demonstrated greater efficacy in these patients is the ROS1/MET tyrosine kinase inhibitor crizotinib. The outcome of the EUCROSS clinical trial was a mean survival rate of 55% at 48 months in patients with NSCLC with ROS rearrangement (57,58). On the other hand, the efficacy of entrectinib, a ROS1/tropomyosin tyrosine kinase inhibitor, has also been demonstrated by different studies. Response rates of 67.1% were obtained, with a response rate of 72.9% for intracranial metastases and a progression-free survival of 15.7 months (59). As with EGFR-TKIs, there are mechanisms of immunoresistance to crizotinib, with lorlatinib exhibiting a greater efficacy in patients who had previously received crizotinib (60). Despite the fact that only a small number of patients are candidates for immunotherapy with ROS1 rearrangement, they obtain very relevant response rates with high average survival rates. Since the vast majority of patients do not carry any driver mutations, other histological

markers are being evaluated with different types of targeted therapies, where the programmed death receptor (PD) and its ligands PDL1 and -2 stand out, which act as inhibitory factors of the immune response (61). The expression of PDL1 has been demonstrated in different tumors and authors have described its expression level by immunohistochemistry in NSCLC. Aggarwal *et al* (62) analyzed 4,784 patients and observed that 28% had a PDL1 expression of ≥50%, 38% had PDL1 expression between 1 and 49 and 33% had an expression of <1%. Of these, 80% corresponded to the non-squamous variant (mainly adenocarcinoma) and 20% to the squamous variant (62). Treatment targeting PDL1 expression is based on several clinical trials, including KEYNOTE-189, KEYNOTE-407, KEYNOTE 024 or IMpower 110. According to the results of the previous clinical trials, in patients with PDL1 expression <50% or unknown metastatic status, the combination of pembrolizumab (anti-PD1) plus pemetrexed and carboplatin in non-squamous NSCL are recommended (63,64). In those patients with >50% expression of PDL1 who do not have any rapidly progressive disease, it is recommended as a first-line treatment monotherapy with pembrolizumab or atezolizumab (anti-PDL1) (65,66). In cases of rapidly progressive disease, it is recommended to start pembrolizumab plus chemotherapy (67). Immunotherapy may be applied based on different molecular alterations and a large therapeutic arsenal is available, which allows clinicians to offer new therapeutic opportunities to patients diagnosed with NSCLC.

In addition, the use of molecular markers has made it possible to better classify the subtypes of lung cancer in cases where the morphology is not clear or when biopsies are not able to be performed to obtain the necessary material for diagnosis. For instance, markers such as thyroid transcription factor 1, which is associated with the EGFR mutation, napsin A and surfactant A, are much more specific for lung adenocarcinoma, while p63, cytokeratin 5/6, SOX2 and desmoglein-3 are characteristic of squamous cell carcinoma (68). Likewise, there are immunohistochemical markers related to a greater extent with SCLC, such as LMWK, CAM5.2, chromogranin or CD56, among others (69).

Collectively, the prognostic and therapeutic implications of driver mutation analysis have led to the creation of a wide therapeutic armamentarium even in those cases with rare genetic alterations, which reinforces the importance of molecular biology in the treatment of lung adenocarcinoma. In conclusion, the importance of driver mutations as biomarkers and their molecular analysis have achieved an improvement in the survival of patients with lung adenocarcinoma in recent years, allowing to guide the use of therapeutic agents to reduce the complications associated with chemotherapy treatment and improve the quality of life of patients.

3. Serological markers

Even though lung adenocarcinoma is one of the main neoplasms in the current population and despite the numerous driver preferences that have been described, this type of cancer currently lacks effective screening programs. The imaging screening test is based on the use of low-radiation CT of the chest, the implementation of which, notwithstanding having demonstrated its usefulness, is limited in daily clinical

practice (70). In addition, the presence of solitary pulmonary nodules in the general population is observed in up to 13% of chest CT scans, of which ~23% may be due to malignant pulmonary neoplasms. The evaluation of these solitary pulmonary neoplasms may involve the use of invasive tests, which are not exempt from complications (71). Furthermore, as mentioned above, the majority of patients with lung adenocarcinoma are diagnosed at an advanced stage of the disease, so the implementation of serological markers may provide noteworthy benefits in the early diagnosis of the disease.

In this sense, various authors have attempted to define the clinical usefulness of potential serum biomarkers. CEA is one of the most studied markers in lung cancer, being the tumor serological marker that is mainly used for follow-up and diagnosis in colorectal cancer. Grunnet and Sorensen (72) evaluated the usefulness of CEA in lung adenocarcinoma in 217 studies where is observed a correlation with elevated serum level of CEA and risk of recurrence and death in this neoplasm. The diagnostic utility of CEA as a serological marker has also been evaluated together with other potential serum biomarkers. In this sense, Xu et al (73) defined the sensitivity and specificity of CEA, neuron-specific enolase (NSE) and matrix metalloproteinase-9 in 36 patients with lung cancer, obtaining an area under the curve (AUC) of 0.84, 0.8 and 0.89, respectively. In the receiver operating characteristics analysis, the AUC is a measure of the diagnostic performance of a test by comparing sensitivity and specificity, with a value of 1 being the highest score attainable. Despite this, Yang et al (74) obtained AUCs that did not exceed 0.65 when markers such as CEA, CA125, CY211 (cytokeratin 19 fragment), NSE or squamous cell carcinoma antigen (SCC) were used independently in 2,063 individuals, of whom 780 were healthy controls, 650 patients had pneumonia and 633 patients had lung cancer to differentiate lung cancer from patients without cancer. Conversely, these authors also evaluated the combination of different markers to increase the AUC. For instance, the use of markers such as CEA+CA125+CY211+SCC reaches an AUC of 0.867 when comparing patients with lung adenocarcinoma vs. healthy patients. The limited utility of the markers to be used individually relies on the fact that numerous benign entities, such as bronchiectasis, pneumonia or chronic lung diseases, may raise different markers on their own. For this reason, combinations of serological markers have only reached AUCs of >0.715 in differentiating pneumonia from pulmonary neoplasia (74).

The association between serological markers and histology in lung adenocarcinoma is limited and histological confirmation would always be necessary to analyze driver mutations for therapeutic planning of patients. In 155 patients with lung adenocarcinoma, Gao et al (75) examined how the serological CEA concentration is related to neoplastic lesions that carry EGFR mutations, indicating that this was associated with an unfavorable prognosis and progression during the use of EGFR inhibitors (75). In this light, other studies such as that by Molina et al (76) indicated the usefulness of serological markers in combination, such as CEA, CA15.3, SCC, CY 211, NSE and progastrin-releasing peptide, in the initial diagnostic of 3,144 individuals with suspected lung cancer, of which the diagnosis of neoplasia was confirmed in 1,828. They reported a sensitivity of 88.5% and a specificity of 82%, in addition to a positive predictive value of 87.3%, therefore demonstrating

the relevance of serological markers in the early diagnosis of this pathology. It should be noted that in patients with nodules <3 cm, the negative predictive value was 71.8%. In other words, 71.8% of the patients who did not have any elevated serological markers did not have a true malignant neoplasm, which allows for more conservative management, limiting the use of aggressive thoracic surgeries and biopsies (76). The prognostic utility of different serological markers in lung cancer has been evidenced by different authors. For instance, Chen et al (77) evaluated the preoperative levels of the serological markers CEA, CYFRA21-1, NSE, CA 19-9, CA 153 and CA125 in 2,654 patients with NSCLC who were candidates for resection surgery, demonstrating how high levels of CEA, CYFRA 21-1 or CA125 are associated with unfavorable survival and higher rates of recurrence. In this light, Bes-Scartezini and Saad Junior (78) determined that in 112 patients with non-squamous NSCLC, elevation of CA125 or Ca15-3 was associated with unfavorable survival (77,78).

Therefore, in recent years, the use of serological markers has not been applied in daily clinical practice, despite the fact that combination panels have demonstrated their usefulness not only in the initial diagnosis of these patients, but also in the follow-up or evaluation and prognosis, acting as potential prognostic and predictive biomarkers.

4. CTCs

The concept of CTCs is based on the existence of epithelial cells in the blood circulatory system after a process of angioinvasion and metastatic dissemination, which are not normally seen in patients without cancer. Usually, 1 CTC may be found for every 10 million leukocytes in peripheral blood. There are non-tumor conditions in which CTCs are present, generally due to inflammatory diseases such as Crohn's disease or endometriosis, but to a lesser extent due to tumor processes (79). The relevance of CTCs has already been described in prostate, breast and colon cancer, where their presence is accompanied by a worse prognosis and higher rates of recurrence after chemotherapy or surgery (80). The identification of driver mutations by different techniques has represented a real advance in the treatment and prognosis of patients with NSCLC. It is important to remark that patients may acquire new driver mutations during targeted therapies, hence developing therapy resistance, which would require a re-biopsy in most patients. Conceptually, rebiopsy would be useful not only to study the mechanisms of immunoresistance (such as the T790M mutation in patients with EGFR-mutated lung adenocarcinoma), but also to understand the underlying pathophysiology of the molecular pathways that ultimately cause immunoresistance (81). The utility of peripheral blood liquid biopsy would allow the detection of CTCs in order to study new therapies and develop cell cultures to facilitate a deeper understanding of the physiological mechanisms of the metastatic process (82). Simultaneously, CTCs also allow the monitoring of immunochemotherapy treatment, since a decrease in these cells would indicate a better response to the therapies received (83).

Multiple methods have been studied for the detection of CTCs. The gold standard method approved by the FDA is based on the detection of the epithelial proteins epithelial

cell adhesion molecule (EpCAM), cytokeratins 8, 18 and or 19 using the Cellsearch method, which is approved for metastatic breast cancer, prostate adenocarcinoma and colorectal cancer (84-86). With respect to lung cancer, numerous authors have used the Cellsearch method to detect CTCs. In this sense, the detection of CTCs has been observed in both NSCLC and SCLC. Hou *et al* (87) studied these cells in 97 patients with SCLC, detecting CTCs in 85% of them. Furthermore, the average survival of patients with >50 CTCs per 7.5 ml of blood was limited to 5.4 months, while in patients with <50 CTCs, a higher median survival was reported (87). This is in consonance with the results of Naito *et al* (88), who reported that 51 patients with lung cancer with elevated CTCs had unfavorable survival. Regarding lung adenocarcinoma, the role of CTCs was also demonstrated in the diagnosis and monitoring of these patients. Indeed, previous works have been able to detect ALK rearrangements in CTCs, which may allow starting therapy with tyrosine kinase inhibitors such as crizotinib in the future, demonstrating its usefulness in the follow-up of patients diagnosed with lung cancer (89,90). Other methods for the detection of CTCs, such as positive immunoselection of EpCAM, negative immunoselection of leukocytes, filtration, immunomagnetics, electrophoresis or flow cytometry have also demonstrated their utility but are not currently approved by the FDA, as they are based on complex techniques that require very well-trained personnel, which may not be accessible in daily clinical practice (84). In reference to liquid biopsy using CTCs, the main limitations of this technique are mainly based on sample collection and processing techniques, given that CTCs may become fragile and cannot be processed properly. In addition, its high price and technical complexity must be highlighted, which frequently requires a support laboratory that not all hospitals are able to afford. All of this may affect the diagnostic performance, decreasing both sensitivity and specificity, and since these techniques have diagnostic-therapeutic repercussions, they must be validated in large clinical trials. Therefore, despite the immense benefits in the detection of CTCs, these techniques are accompanied by limitations that may restrict their use in real clinical practice (91).

Therefore, in recent years, advances in the genetic analysis of lung cancer have revolutionized the treatment and management of this disease. The possibility of obtaining the necessary material through liquid biopsy in peripheral blood and the possibility of evaluating the immunohistochemical and genetic expression of CTCs is accompanied not only by an improvement in the diagnosis of this disease, but also in the monitoring and early detection of mechanisms of immunoresistance that may cause a fatal outcome of these patients.

5. MicroRNAs

MiRNAs are small non-coding RNA molecules with a length of ~20 nucleotides that regulate the post-transcriptional expression of genes that may be related to cell differentiation, proliferation and apoptosis processes by promoting or suppressing the expression of a gene after transcription. A miRNA molecule regulates the post-transcriptional expression of up to 200 different genes and its study may expand the understanding of the underlying pathophysiology of the metastatic process (92). In relation to lung cancer, the implications of miRNAs are numerous-they may promote processes such as cellular proliferation, metastatic invasion and therapy resistance through the upregulation or downregulation of either tumor suppressor genes or oncogenes (93). As previously mentioned, tobacco is the main cause of lung cancer in the general population and it has been indicated how the levels of miR-532-5p, miR-25-3p and miR-133a-3p were significantly higher in patients with lung carcinoma compared to healthy controls, also observing differences between expression levels depending on the smoking status (94). On the other hand, Nymark *et al* (95) indicated how numerous miRNAs are dysregulated according to exposure to asbestos and its relationship with lung cancer. Likewise, there are numerous miRNAs that have been implicated in EGFR mutations, such as miR-7, miR-27a-3p, miR-30 and miR-34, which led to the activation of the RAS/MEK and PI3K/mTOR pathways with consequently uncontrolled cell proliferation (96,97). Similarly, a role of miR-96 has been described in the altered levels of ALK and the activation of the RAS/MEK and PI3K/mTOR metabolic pathways (98). miR-760 causes alterations in the expression of ROS1, while let-7, miR-193a-3p or miR-148a-3p are related to KRAS mutations, previously demonstrating their importance in tumor progression (99-102).

The diagnostic utility of miRNAs has also been studied by different authors. For instance, the presence of miR-205 was specific for squamous cell carcinoma compared to miR-124a, which is more characteristic for lung adenocarcinoma (103,104). The relationship between histology and miRNA expression has made it possible to demonstrate how miR-93, miR-221 and miR-30e are specific for squamous cell carcinoma, while miR-29b, miR-29c, let-7e and miR-125a-5p are more specific for lung adenocarcinoma (105). One of the uses of miRNAs is based on the possibility of them being used in screening programs that determine the blood levels of multiple miRNAs, which simplifies the diagnostic process as well as its ease of performance and improves its diagnostic performance. Studies such as that by Montani *et al* (106) have evaluated the use of a kit with 34 miRNAs in 1,115 individuals with a high risk of lung cancer, obtaining a sensitivity of 75.9%, a specificity of 77.8% and an AUC for the diagnostic yield of 85% (106). These results are in agreement with those obtained by Sozzi *et al* (107), who analyzed 69 patients with lung cancer using a kit of 24 miRNAs, obtaining a sensitivity of 87% and a specificity of 81% (107). Asakura *et al* (108) reported that after analyzing up to 2,588 miRNAs in 208 patients with lung cancer compared to healthy controls, the highest diagnostic yield using miRNAs was obtained with miR-1268b and miR-6075, obtaining a sensitivity and specificity of 99% and an AUC of 0.993 for lung cancer screening (108).

On the other hand, the expression of different miRNAs has been studied to analyze its relationship with the prognosis of patients. Xiao *et al* (109), in a meta-analysis of 15 studies that included a total of 1,753 patients with both SCLC and NSCLC, described that upregulation of miR-125b, miR-21, miR-141, miR-200c, miR-197, miR-41, miR-370, miR-376α, miR-192 and miR-662 and the downregulation
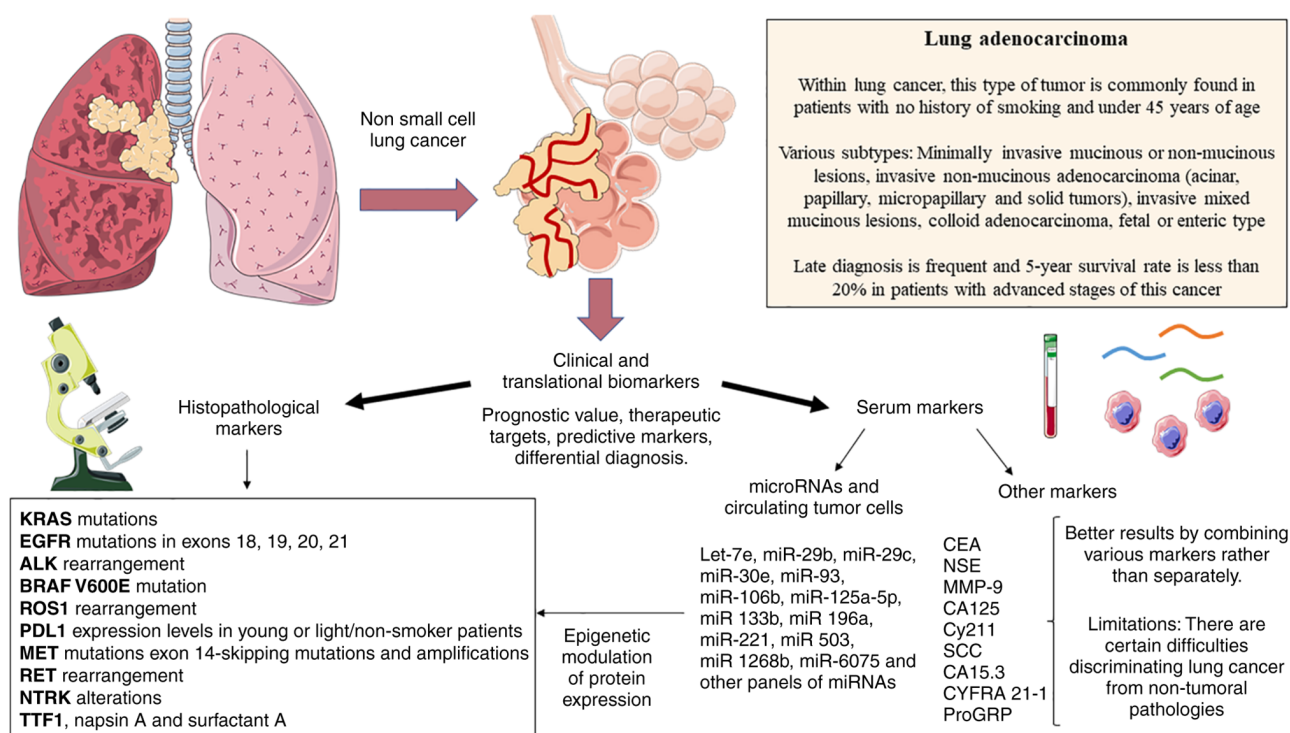
Figure 1. Main clinical and translational biomarkers studied in lung adenocarcinoma. Both histopathological and serological markers are available. All of these markers are involved or appear as a consequence of the tumoral biology, aiding in the development of novel therapeutic strategies, prognostics, response to therapy prediction or in differential diagnosis. miRNA/miR, microRNA; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; MET, mesenchymal epithelial transition factor receptor; RET, rearranged during transfection; BRAF, V-Raf murine sarcoma viral oncogene homolog B; NTRK, neurotrophic tyrosine receptor kinase; CEA, carcinoembryonic antigen; CA125, cancer antigen 125; CY211, cytokeratin 19 fragment; SCC, squamous cell carcinoma antigen; NSE, neuron-specific enolase; ProGRP, pro-gastrin-releasing peptide; TTF1, thyroid transcription factor 1; ROS1, c-ros oncogene 1; PDL1, programmed death ligand 1.

of miR-26b, miR-381, miR-146α, miR-148α, miR-204, miR-374α, miR-638 or miR-148b were associated with poor median survival, evidencing the complex role of miRNAs in lung cancer.

It should be noted that alterations in different miRNAs have been related to mechanisms of chemoresistance and sensitivity to immunotherapy. For instance, overexpression of miR-106b leads to a decrease in the P-glycoprotein responsible for chemoresistance mechanisms to cisplatin, which causes greater sensitivity to cisplatin (110). In turn, Qiu et al (111) have demonstrated that downregulation of miR-503 alters the expression of proteins related to chemoresistance processes, such as the antiapoptotic protein Bcl-2, while another study indicated that overexpression of miR-196a leads to decreased efficacy of cisplatin (112). Given that in recent years, immunotherapy has laid a foundation for the management of patients with lung cancer, the expression of miRNA in this context has been evaluated in numerous studies. For instance, Bisagni et al (113) examined 32 patients with lung adenocarcinoma receiving second- or third-line treatment with erlotinib, an EGFR tyrosine kinase inhibitor, and miR-133b upregulation was associated with better progression-free survival. However, the main limitation of miRNAs in lung cancer in terms of their usefulness for screening is their limited specificity. For instance, miR-21-5-p, miR-155-5p and miR-210-3p are expressed in different neoplasms, such as breast or colon cancer, among others, which would require patients to undergo multiple diagnostic tests with

the probability of adverse effects without a clear diagnostic suspicion. In addition, both upregulation and downregulation of the same miRNA may be observed in different neoplasms, which increases the diagnostic uncertainty. In addition, large clinical trials should be implemented to specifically validate detection kits that are cost-efficient in different neoplasms so that they may be systematically applied in different malignant neoplasms (114).

Examination of miRNAs, which may be performed by liquid biopsy in peripheral blood, has useful implications in the diagnosis, follow-up and treatment of patients with pancreatic adenocarcinoma that may improve diagnosis in early stages and improve the understanding of the mechanisms of immunochemical resistance in these patients.

6. Conclusions

Lung cancer is one of the most frequent neoplasms and the deadliest type of cancer, which is specifically associated with tobacco consumption. Despite numerous efforts and screening programs that have been performed, most patients are diagnosed in the advanced stages of the disease. Lung adenocarcinoma is a specific subtype of NSCLC with unique histological, radiological, epidemiologica and clinical characteristics. Recent advances in the molecular biology of these tumors have permitted the identification of multiple markers, as summarized in Fig. 1. The study of these markers has allowed the development of numerous

Table I. Main translational applications derived from the potential biomarkers collected in the present study.

Biomarker	Type	Diagnostic/prognostic value	Predictive/therapeutic utility	(Refs.)
EGFR	Driver mutation	15% of adenocarcinomas (up to 50% in Asian population)	EGFR inhibitors	(34,35)
T790M EGFR mutation	Driver mutation	Resistance to first-and second-generation EGFR inhibitors	Response to third generation tyrosine kinase inhibitors	(39,40)
ALK	Driver mutation	~4% of adenocarcinomas, non-smoking young patients	ALK inhibitor	(41-45)
MET	Driver mutation	~6% of adenocarcinomas	MET inhibitor	(46,47)
RET	Driver mutation	1-2% of adenocarcinomas, non-smoking young patients	RET inhibitor	(50,51)
BRAF	Driver mutation	4% of adenocarcinomas	anti-BRAF inhibitor	(52,53)
NTRK	Driver mutation	<1% of adenocarcinomas	Tropomyosin kinase inhibitor	(54)
CEA+CA125+CY211+SCC	Serological marker combination	AUC 86.7%	Screening	(61)
CEA, CA15.3, SCC, CY21, NSE and ProGRP	Serological marker combination	Sensitivity, 88.5%; specificity, 82%; positive predictive value, 87.3%	Early diagnosis	(63)
Circulating tumor cells	Liquid biopsy diagnostic kit	Detectable in up to 85% of small-cell lung cancers	-	(70-72)
miR-93, miR-221 and miR-30e	miRNA	Specific for squamous cell lung cancer	-	(90)
miR-29b, miR-29c, let-7e and miR-125a-5p	miRNA	Specific for lung adenocarcinoma	-	(90)
Panel of 34 miRNAs	miRNA kit evaluated in 1115 individuals	Sensitivity, 75.9%; specificity, 77.8%; AUC, 85%	-	(84)
Panel of 24 miRNAs	Evaluated in 939 individuals	Sensitivity, 87%; specificity, 81%	-	(91)
miR-1268b and miR-6075	miRNA	Sensitivity, 99%; specificity, 99%; AUC, 0.993	-	(92)
miR-106b	miRNA	-	Increased sensitivity to cisplatin	(94)
miR-196a	miRNA	-	Increased resistance to cisplatin	(96)
miR-503	miRNA	-	Less sensitive to chemotherapy	(95)
miR-133b	miRNA	-	Better survival in patients with mutated EGFR	(97)

miRNA/miR, microRNA; AUC, area under the receiver operating characteristic curve; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; MET, mesenchymal epithelial transition factor receptor; RET, rearranged during transfection; BRAF, V-Raf murine sarcoma viral oncogene homolog B; NTRK, neurotrophic tyrosine receptor kinase; CEA, carcinoembryonic antigen; CA125, cancer antigen 125; CY211, cytokeratin 19 fragment; SCC, squamous cell carcinoma antigen; NSE, neuron-specific enolase; ProGRP, pro-gastrin-releasing peptide.

targeted therapies, also aiding to improve the prognosis, diagnosis and prediction of the response to different therapeutic regimes. Likewise, numerous serological markers have been studied, demonstrating promising translational uses. The main markers studied with their most important translational/clinical applications are summarized in Table I. Overall, there is still much to explore in the field of biomarkers in lung adenocarcinoma, particularly regarding the aim to improve early detection of the disease and identifying new molecular routes that may be used for targeted therapies, which is proving to be one of the most important advances in the field of oncology.

Availability of data and materials

Not applicable.

Authors' contributions

MAO, FN, LP, MAM were involved in the conceptualization of the study. MAO and MAM were involved in funding acquisition. MAO was involved in project administration. MAO, FN, LP, OFM, CGM, MAS, MA, JM and MAM were involved in the investigative aspects of the study. MAO, FN, LP, OFM, CGM, MAS, MA, JM and MAM were involved in data validation. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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