Lung adenocarcinoma: from molecular basis to genome-guided therapy and immunotherapy

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Abstract: Although adenocarcinoma (ADC) is the most frequent lung cancer, its diagnosis is often late, when the local invasion is important and/or the metastases have already appeared. Therefore, the mortality at 5 years is still very high, ranging from 51% to 99%, depending on the stage. The implementation of different molecular techniques has allowed genomic studies even in relatively small histological samples such as obtained with non-invasive or minimally invasive techniques, facilitating a better phenotyping of lung ADC. Thus, current classification differentiates between preinvasive lesions (atypical adenomatous hyperplasia and in situ ADC), minimally invasive ADC (MIA) and invasive ADC. ‘Field cancerization’ is a concept that refers to progressive loco-regional changes occurring in tissues exposed to carcinogens, due to the interaction of the latter with a predisposing genetic background and an appropriate tissue microenvironment. Somatic genetic alterations, including mutations but also other changes, are necessary for oncogenesis, being especially frequent in lung ADC. Changes in the epidermal growth factor receptor (*EGFR*) gene, Kirsten rat sarcoma viral oncogene (*KRAS*), v-Raf murine sarcoma viral oncogene homolog B (*BRAF*), gene encoding neurofibromin (*NF1*), anaplastic lymphoma kinase (*ALK*) and *ROS1* are the main genes that suffer alterations in the tumors of patients with ADC. Molecular profiling of these tumors allows more targeted treatments through two distinct strategies, genome-guided therapy and immunotherapy. The former, targets the aberrant pathways secondary to the genomic alteration, whereas the latter may be based on the administration of antibodies [such as those against cytotoxic T-lymphocyte antigen 4 (CTLA-4) or the programmed cell death ligand 1/protein 1 pathway (PD-L1/PD-1)] or the stimulation of the patient’s own immune system to produce a specific response. These strategies are obtaining better results in selected ADC patients.

Keywords: Lung cancer; genome-wide association studies (GWAS); adenocarcinoma classification; carcinogenesis; genome-guided therapy; immunotherapy

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Introduction

Lung cancer is the second most frequently diagnosed tumor in both men and women worldwide, and is the leading cause of cancer-related deaths. In the United States it represents 14% of all neoplasms and is estimated to have produced more than 150,000 deaths in the last year (1,2). This epidemic burden began around the mid-20th century, when the mass-production of packet cigarettes became extended in Western Europe and the United States. Tobacco smoke is the main factor for lung cancer, since it is accepted that it accounts for 80% in males and at least 50% in females. However, although the etiological role of tobacco is crucial, up to 25% of lung cancer presents in people that have never smoked. This is especially evident in women with the adenocarcinoma (ADC) subtype. In these cases, other risk factors such as air pollution, environmental and work related carcinogens also seem to play an important role (3-5). There are two main histological types of lung tumors: small-cell lung cancer (SCLC), and non-small-cell lung cancer (NSCLC). The latter represents 80–85% of these tumors, and different histological subtypes can be distinguished: squamous cell carcinoma (SqCC) (44% in men and 25% in women), pulmonary ADC (28% in men and 42% in women) and large-cell and undifferentiated carcinomas (around 9%); rare subtypes accounting for less than 1% of the cases. The dominant histological type strongly varies depending on the smoking status, ethnic background and geographic location, but nowadays it is accepted that the most frequent is ADC, especially in Asian women (more than 70% in Japanese females) (6,7). Even though the incidence rate of lung cancer has been declining in men since the 1980s and in women since the mid-2000s, and that major efforts have been made in research, smoking prevention, early detection and global healthcare approaches, there have still been no overall significant changes in 5-year survival in the last three decades. Moreover, the 1- and 5-year survival rates in lung cancer are 44% and 17%, respectively, and even in patients with a very early stage disease, when supposedly curative surgery is performed the 5-year survival is less than 60% (2,8).

The use of next-generation sequencing (NGS) technologies has confirmed the prevalence of somatic driver alterations in more than 70% of pulmonary ADC. In fact, the Cancer Genome Atlas (TCGA) has identified that 35% of patients have mutations in oncogene *TP53* (tumor suppressor gene 53), overlapping with oncogenic driver alterations such as mutations in *KRAS* (Kirsten rat sarcoma viral oncogene), *EGFR* (epidermal growth factor

receptor 1 or ErbB1 tyrosine kinase receptor oncogene, also denominated *ErbB1* or *HER1*), *BRAF* (v-Raf murine sarcoma viral oncogene), *MET* (mesenchymal-epidermal transition oncogene, encoding a tyrosine kinase receptor), *ERBB2* (epidermal growth factor receptor 2 oncogene, also encoding a tyrosine kinase receptor, called ErbB2 or *HER2* as well), and fusions in *ALK* (anaplastic lymphoma kinase), *ROS1* (encoding tyrosine-protein kinase ros) or *RET* ('rearranged during transfection', codifying a tyrosine kinase receptor) oncogenes, all of them with potential therapeutic implications. Moreover, in recent years new guided therapies have already appeared that are modifying the prognosis of selected groups of patients who have somatic driver alterations. In contrast with ADC, although *TP53* mutations are reported in as much as 81% of SqCC, targetable driver somatic alterations are not frequently found in this tumor subtype (9,10).

Genetic risk factors of lung cancer

As previously mentioned, tobacco smoking is the main risk factor for lung cancer, but there is also an important percentage of never-smokers who develop this tumor. For instance, in the USA, 17,000–26,000 annual deaths can be attributed to lung cancer in never smokers. Thus, environmental carcinogens also seem to play an important role. These external factors appear to combine with genetic susceptibility. In this regard, studies performed both in smokers and never-smokers strongly suggest that polymorphisms in genes involved in DNA repair, cell-cycle regulation, apoptotic pathways, inflammation and telomere length are related with lung cancer (11-14). However, mutation in tumor-suppressor genes also seems to modify susceptibility to this tumor. Both *TP53* and *TP63* mutations have been reported in patients with either ADC or SqCC. Interestingly, when a tumor-suppressor gene is mutated the risk of multiple neoplasms (including lung cancer) becomes increased. For instance, in the Li-Fraumeni Syndrome, a dominant autosomal disorder, more than half of the affected families have inherited mutations in the *TP53* gene and patients present multiple neoplasms in childhood and adolescence. If they survive until adulthood, the risk of tumors, including lung cancer, is highly increased. In turn, *TP63* that encodes p63 (tumor suppressor or transformation-related protein 63) is also associated with lung cancer, especially in never-smoker females in Asia (15,16).

Genome-wide association studies (GWAS)

GWAS are population-based studies used to identify single-nucleotide polymorphisms (SNPs) in different genetic loci. The purpose of these genome-wide investigations is to find genetics alleles that are associated with disease phenotypes. At least 28 SNPs have already been observed to be significantly associated with a risk of NSCLC. Of them, three major loci strongly relate to lung cancer: these are 15q25 of the genes encoding neuronal nicotinic acetylcholine receptor (nAChR) (subunit genes *CHRNA3* and *CHRNA5*), 5p15 (*TERT* and *CLPTM1L*, genes encoding telomerase reverse transcriptase and cleft lip and palate transmembrane 1-like protein, respectively), and 6p21 (*BAT3* or *HLA-B* associated transcript 3 and *MSH5* or *MutS Homolog 5* genes, codifying for large proline-rich protein and a MutS protein involved in DNA repair, respectively). These associations are particularly related to lung cancer in specific ethnic groups, such as Caucasians and Asians (17-19). However, in the vast majority of GWAS, SNPs have demonstrated a strong correlation of polymorphism in two genes, those encoding *TERT* and *CLPTM1L*, with lung cancer, indifferently of the ethnic origin of the patients. In particular, *TERT* polymorphisms are especially associated with ADC in never-smokers. Moreover, GWAS strongly suggest that both *TERT* and *CLPTM1L* polymorphisms actually modify the susceptibility to further develop a lung cancer (20-24).

Updated pathological classification of ADC

ADC has become the most common histological subtype of lung cancer in most countries. In 2011 the International Association for the Study of Lung Cancer (IASLC), the American Thoracic Society (ATS) and the European Respiratory Society (ERS) published a proposal of ADC classification that was finally included unchanged in April 2015 in the 4th edition of the WHO Classification of Tumors of the Lung, Pleura, Thymus and Heart (25). Previous editions based the diagnosis of lung cancer on routine histological criteria obtained from resection samples, but the new classification also integrates immunohistochemistry, and gives specific terminology and diagnostic criteria to smaller biopsies and cytology samples. These criteria would be very helpful for clinicians and patients since around 70% of lung cancers are detected now in advanced stages being unresectable. Moreover, patients would be treated with more personalized chemotherapy

and/or radiotherapy with the use of the new criteria. Thus, it is very important to differentiate between ADC and other lung tumors, even in small biopsy specimens. Many tumors show clear morphologic features, but if the sample showed no clear squamous or glandular features, a minimal immunohistochemical workup with specific markers would make the difference. At the moment, TTF-1 (thyroid transcription factor 1) and p40 (which recognizes the Δ Np63-a p63 isoform) are the best markers for ADC and SqCC, respectively (6,25-30).

The new ADC classification has interesting innovations. For instance, the term bronchioloalveolar carcinoma (BAC) is no longer used. However, tumors formerly named mucinous BAC are now classified as invasive mucinous ADC, whereas the new name for previously called non-mucinous BAC is lepidic-predominant ADC (25). There is also a new subtype called micropapillary ADC, which has a poorer prognosis. In addition, there are new terms such as AIS (*'in situ'* ADC) and minimally invasive ADC (MIA). Moreover, comprehensive histological subtyping based on the predominant subtype is recommended for invasive lung ADC, and the term "mixed subtype" is not used anymore.

Preinvasive lesions

Atypical adenomatous hyperplasia

This is a small (usually 0.5 cm or even less) atypical proliferation of type II pneumocytes along preexisting alveolar walls, which resembles but falls short of diagnostic criteria for non-mucinous AIS. Atypical adenomatous hyperplasia is most commonly diagnosed as an incidental histologic finding, which is present in 5–20% of lung cancer resection specimens. The appearance of this atypical proliferation in CT scan is the presence of small ground glass nodules of 5 mm or less (25).

In situ ADC (AIS)

This has been considered as a preinvasive lesion in the new ADC classification since it grows purely with a lepidic pattern without invasion. Most of the cases are non-mucinous, with a proliferation of type II pneumocytes or club cells (formerly denominated 'Clara cells'). More rarely they may be mucinous, with tall columnar goblet cells and abundant mucin in the apical end. The typical image of non-mucinous AIS in the CT scan is to observe small ground glass nodules, whereas the mucinous subtype often has the form of a solid nodule (25). It is worth noting that if AIS is completely resected, the 5-year disease-free survival reaches 100%.

MIA

This concept was introduced to define a relatively benign form of ADC, with nearly a 100% 5-year disease-free survival. MIA refers to a small (≤ 3 cm) solitary ADC with predominant lepidic growth having an invasion of 5 mm or less. Most of these tumors are non-mucinous, although the mucinous form also exists. Similarly to AIS, while the non-mucinous MIA typically shows ground glass nodes in the CT scan (with a solid component measuring 5 mm or less), the mucinous tumor presents as a solid nodule (25).

Invasive ADC

Invasive ADC is classified according to predominant findings. For this, the use of a comprehensive histological subtyping is mandatory, since it allows the estimation of the percentages of the different components. The latter is currently expressed in a semi quantitative fashion, with 5–10% increments. Tumors of mixed characteristics but containing a predominant lepidic growth pattern of type II pneumocytes and/or club cells (formerly known as non-mucinous BAC), which have an invasive component >5 mm are considered as ‘lepidic predominant ADC’. Moreover, as previously mentioned a micropapillary predominant subtype has been added to the new classification. The signet ring and club cell carcinoma subtypes are characterized by a relatively high percentage of these features. Although the latter are commonly observed in the solid subtype, they can also show acinar or papillary patterns. Interestingly, there is a good correlation between the amount of the ground glass and the solid component in the CT, and the lepidic growth and the invasion of the tissue, respectively (25).

ADC variants

The variants of lung ADC accepted today are invasive mucinous, colloid, fetal and enteric ones. The invasive mucinous ADC (formerly known as mucinous BAC) frequently associates *KRAS* mutation and lack of TTF-1, and is also characterized by multicentric lung lesions. Histologically, these tumors show different amounts of lepidic, acinar, papillary or micropapillary growth modalities, all of them characterized by the already mentioned columnar cells with abundant apical mucin and small base-oriented nuclei. In this case, the CT scan frequently shows localized or multifocal consolidation, conforming nodules or lobar involvement, as well as air

bronchogram (25).

Carcinogenesis and cancer hallmarks

Field change cancerization

Field ‘cancerization’ or ‘effect’ denotes a large variety of loco-regional changes occurring on the surface of tissues that are exposed to carcinogens for a relatively extended period. These cellular and molecular changes, in otherwise apparently healthy cells, predispose to the occurrence of cancerous lesions. The lung, and especially the bronchial epithelium, is a perfect example of field cancerization. A predisposing genetic background along with long-term exposure to tobacco and/or environmental carcinogens, and an appropriate lung tissue microenvironment result in a field susceptibility that could trigger cancer initiation, evolution and progression (31,32).

Epigenetic changes

Epigenetic changes are heritable modifications that affect gene expression and other DNA dependent processes without actually changing DNA sequence (33). Although genetic changes play an essential role in ADC tumorigenesis, epigenetic modifications are also linked to the genesis and progression of cancer, as well as to the response to chemotherapy. These modifications include DNA methylation, and changes in microRNA-mediated regulation and the histone/nucleosome (34). Moreover, different studies have shown a direct association between the presence of methylation of tumor suppression genes and the prognosis of resectable early stage NSCLC. Recently, Daugaard *et al.* using DNA microarrays, have identified and validated 15 differentially methylated regions (DMRs) in lung ADC, which are absent in the tumor-adjacent normal lung tissue. This study suggests that these DMRs can be used as ADC biomarkers and eventually as targets for novel treatments (35,36).

Hallmarks of cancer

At the beginning of this millennium, Hanahan and Weinberg described the ‘Hallmarks of Cancer’ as the traits that normal cells slowly acquire in their transformation process to a tumor (37). These authors tried to resume the complexity of this process using a multi-hit model, where different characteristics and discrete genetic alterations

Figure 1 Hallmarks and biological events present in cancer.

progressively add up until cancer finally develops. Initially, six hallmarks were described, along with two other emerging findings and two more enabling characteristics that facilitate tumor growth and metastatic dissemination (Figure 1 and Table 1).

Genomic alterations in lung ADC

As already mentioned, the multi-hit and multi-step cancerogenesis model implies that patients with an intrinsic susceptibility (epigenetic modifications or genome heritable traits) exposed to deleterious factors and with an “appropriate” tumoral-peritumoral environment are predisposed to gain specific somatic genetic alterations (see next section) that trigger an initial clonal cell expansion. At the same time, the aforementioned processes continue to add hallmarks and potentiate an abnormal cell proliferation. This dynamic model conceptualizes cancer as an evolutionary process, where a single cell acquires ‘advantageous’ genomic alterations, allowing itself to proliferate without control, invade and metastasize.

Somatic alterations in cancer genome

Genetic alterations are necessary for oncogenesis. Moreover, all malignant cells show DNA modifications at some point during abnormal proliferation. Although these alterations, which are intrinsic to cancer, can be inherited, most of them are the result of errors when DNA becomes copied during cell cycle. In adulthood, DNA has been copied around 30 trillion times, and a cancer-related mutation can occur at any time, with the probability increasing with the passing of years. These acquired changes in DNA are known as ‘somatic mutations’ or, using a better expression ‘somatic genomic alterations’ (since not all the DNA modifications are mutations). However, not all these changes are related with the development of cancer. Those somatic genomic alterations that are actually involved in carcinogenesis are known as “driver” alterations, whereas those that are not, are called “passenger” alterations (46,47) (Figure 2).

Both pulmonary ADC and SqCC have a high mutational burden compared with other cancers. Interestingly, mutated oncogenes considered as therapeutically targetable predominate in the former. Moreover, when the whole

Table 1 Biological hallmarks in lung cancer

Hallmark	Normal cells	Cancer cells	Therapeutic targeting
Sustaining proliferative signaling	Cell division starts when intercellular proliferative signals are released (only when needed)	Proliferative signals constantly being used to form rapidly growing tumor structures	<i>EGFR</i> inhibitors
Evading growth suppressors	Use growth suppression signals to inhibit unwanted proliferation	Suppressors are repressed and continue to grow out of control	Cyclin-dependent kinase inhibitors
Inducing angiogenesis	Vascular endothelial growth factor (VEGF) is released to generate new vessels but only if more nutrients are needed	Unlimited growth implicates a high increase on nutrient demands, and VEGF release is increased	Inhibitors of VEGF signaling
Enabling replicative immortality	Limited replication is done by progressive and accumulative loss of telomeres in each cell division	Telomerase production allows telomere replication, which in turn results in infinite replication	Telomerase Inhibitors
Resisting cell death	Programmed (apoptosis) and necrotic cell death, eliminates cells with a damaged DNA	Apoptosis is attenuated, producing increased cell proliferation, cancer progression and resistance to therapy (38,39)	Pro-Apoptotic BH3 mimetics
Activating invasion & metastasis	Organized growth with differential limits	Tissue barriers are broken and the tumor can invade other organs or vascular and lymphatic vessels (to migrate to other organs)	Inhibitors of HGF/c-Met
Avoiding immune destruction	T-lymphocytes look for surface markers to detect abnormal cells and destroy those with an aberrant behavior	The immune system can be evaded by multiple pathways, mainly avoiding the expression of cell markers	Immunotherapy
Deregulation of cellular energetics	Oxygen obtained from blood supply is used to convert glucose to energy	Higher and unreachable nutrient supply is needed. Anaerobic glucose metabolism occurs	Aerobic Glycolysis Inhibitors
Enabling characteristics			
Tumor-promoting inflammation	Equilibrium between nutrients, inflammatory cells and free radicals is required to produce optimal conditions for normal cell growth and replication	Inflammation modifies cell proliferation, survival, apoptosis and angiogenesis, facilitating the release of reactive oxygen species, promoting carcinogenesis and favoring metastasis (40-43)	Selective anti-inflammatory drugs
Genome instability & mutation	Progressive addition of different hallmarks	Gain susceptibility to both genomic alterations and the appearance of driver mutations. These genomic changes contribute to the multi-step (or multi-hit) process of carcinogenesis (44,45)	PARP inhibitors

exome of twelve different cancers was sequenced, more than 75% of pulmonary ADC showed driver genomic alterations (48). The frequency of these driver alterations can vary depending on the ethnicity, sex or smoking status, but no differences can be found in different lung ADC stages (49). *Table 2* lists the most frequent driver alterations according to TCGA data and the cBioPortal for Cancer

Genomics software (open source) (9,50,51).

Epidermal growth factor receptor (EGFR) gene mutations

EGFR is one of the most studied oncogenes related to lung ADC, being located on the short arm of chromosome 7. The *EGFR* family encodes proteins that belong to the cell-

Figure 2 Passenger and driver genetic abnormalities occurring in carcinogenesis.

surface tyrosine kinase receptor family, and consists of four members: *EGFR* (*HER1* or *ErbB1*), *HER2* (*ErbB2*), *HER3* (*ErbB3*) and *HER4* (*ErbB4*) (52-55). These act as transmembrane glycoproteins, and regulate multiple cell processes including apoptosis, cell motility, angiogenesis and proliferative signaling, and also have an impact on carcinogenesis at multiple levels (56,57). *EGFR* is mutated in 10–16% of ADC, with this percentage being much higher in non-smoking women, especially in Asians (where it reaches a frequency of more than 60%) (58-60). Two different somatic alterations account for more than 90% of the total. One is the L858R mutation (substitution of arginine for leucine at codon 858 in exon 21), which represents 45–50% of the cases, and the other is the E746_A750 deletion (in exon 19) that occurs in 45% of the subjects. In the early stages of the disease, ADC with *EGFR* somatic alterations has a better prognosis than the “wild-

type” tumor after curative resection. Furthermore, even in advanced ADC the presence of *EGFR* alterations positively changes survival due to the genomic-guided therapy with *EGFR* tyrosine kinase inhibitors (60-64).

KRAS mutations

KRAS is one of the three members of the so-called RAS family, along with *HRAS* and *NRAS*. All of them encode low molecular weight proteins that bind to the Guanosine-Triphosphate (GTP), having crucial roles in monitoring the activity of signaling pathways that control normal cell proliferation (65). Moreover, *KRAS* mutations were the first somatic alterations that were identified in lung cancer, and despite being a potential therapeutic target, their significance in the clinical setting still remains controversial (66).

Table 2 Driver alterations in lung ADC

Genetic abnormality	Frequency (%)	
	TCGA data	cBioPortal data
Mutations		
<i>KRAS</i>	32.2	33
<i>EGFR</i>	11.0	14
<i>BRAF</i>	7.0	10
<i>NF1</i>	8.3	11
<i>MET</i> <i>ex14</i>	4.3	8
<i>RIT1</i>	2.2	2
<i>ERBB2</i>	1.7	1
<i>MAP2K1</i>	0.9	<1
<i>NRAS</i> , <i>HRAS</i>	0.8	<1
Amplifications		
<i>MET</i>	2.2	4
<i>ERBB2</i>	0.9	3
Translocations		
<i>ROS1</i>	1.7	2
<i>ALK</i>	1.3	3–8
<i>RET</i>	0.9	1

Besides, they are also the most common mutations detected in lung ADC (33%), being more frequently detected in older men, smokers, and in large-sized solid tumors and poorly differentiated ADC (67–69). Mutations in codon 12 are the most frequently detected (75% of the total) and result in the substitution of glycine for cytosine (Gly12Cys), valine (Gly12Val) or aspartic acid (Gly12Asp), meanwhile mutations in codon 13 are much less observed (around 7%). Unlike EGFR mutations, those occurring in KRAS are strongly related with a poorer prognosis in both early stages of ADC and advanced disease. Unfortunately, the attempts to use guided-therapies to target this mutation-phenotype have been extraordinarily frustrating up to now (67,70–73).

***BRAF* mutations**

BRAF encodes a protein called B-Raf that constitutes a crucial step in the RAS-mitogen activated protein kinase (RAS-MAPK) signal pathway. *BRAF* mutations are present in 7–10% of patients with pulmonary ADC, and

the vast majority of these mutations are characterized by the substitution of valine by glutamate (Val600Glu or V600E) in exon 15 (74,75). Compared with other lung cancers, *BRAF* mutations are almost exclusive to ADC, although their frequency is low compared with that in other extrathoracic cancers such as melanoma (50–66%) and colorectal carcinoma (>15%). Moreover, this driver mutation is more likely to be observed in smokers and women, and can be targeted by B-Raf protein inhibitors (previously experienced in other cancers). Unlike *EGFR* or *KRAS* alterations, the presence of *BRAF* mutations are not associated with changes in prognosis (65,76,77).

Neurofibromin gene (NF1) mutations

NF1 is an oncogene encoding the neurofibromin protein. This gene is located in chromosome 17 and is composed by 60 exons, making it one of the largest genes in the human genome. This oncogene has been widely described in the context of type 1 neurofibromatosis, and acts as a tumor suppressor with a negative-regulation of the *RAS* oncogene (78,79). Neurofibromin also regulates cell adhesion, migration and survival, producing a proapoptotic effect. Patients with neurofibromatosis type 1 are considered at high risk of developing malignancies. It should be noted that since TCGA data of somatic mutations are available, *NF1* mutation has become a potential therapeutic target both in ADC and SqCC. Patients with lung cancer and *NF1* mutation have a concomitant mutation in *KRAS* in 15% of the cases, but in around 70% exhibit no other somatic alteration. It is worth noting that patients with *NF1* alterations in the tumor and those with *KRAS* abnormalities share similar clinical characteristics and prognosis (80).

***MET* amplifications and mutations**

MET is an oncogene that encodes for the transmembrane *MET* tyrosine receptor kinase, with only one known ligand (the hepatocyte growth factor or HGF). The presence of *MET* alterations has a negative impact on prognosis, since amplifications of this gene are related with resistance to *EGFR*-guided therapy in patients with advanced disease, and a high *MET* oncogene copy number is associated with worse prognosis in patients with localized disease. However, *MET* mutations (mutually exclusive with those occurring in *KRAS-EGFR*), despite being identified with a relative high frequency in ADC, have not been related with an oncogenic potential (54,81–84).

ALK translocations

The *ALK* gene is located on chromosome 2 and encodes a transmembrane tyrosine kinase. Nearly 30 different *ALK* fusions have been described, including the *EML4-ALK* fusion, which is frequently observed in lung ADC (85). This fusion is created by an inversion of the short arm of chromosome 2 that binds exons 1–13 of *EML4* (encoding echinoderm microtubule associated protein like 4) to exons 20–29 of *ALK*, resulting in the synthesis of a chimerical protein with constitutive *ALK* activity (86–88). Patients with *ALK*-rearranged ADC are usually young, never-smokers and women, showing moderately or poorly differentiated peripheral tumors (89,90). In general, *ALK* alterations are mutually exclusive with *KRAS-EGFR* mutations, having prognosis implications due to the impact of guided-therapies (91).

ROS1 translocations

ROS1 is an oncogene that encodes tyrosine kinase receptor, being phylogenetically related to *ALK*. Unlike *ALK* translocations, *ROS1* rearrangements include one of twelve different partner proteins, and in lung ADC its fusion with *CD74* (cluster of differentiation 74), *EZR* (coding for protein ezrin), *SLC24A2* (encoding the sodium/potassium/calcium exchanger 4) or *FIG* (encoding the fused in glioblastoma protein) genes has emerged as a new driver alteration with promising therapeutic implications. In NSCLC patients the presence of a *ROS1*-rearrangement is specific for ADC, being frequently observed in Asiatic young women and never-smokers (92–94).

Molecular profiling in lung ADC: when and how?

The complete genetic profile of lung ADC is not easily available in standard clinical practice due to the needs of relatively large tissue samples, which often involve the use of invasive techniques, as well as a good molecular biology laboratory, with properly trained personnel, and the elevated costs of the procedure. For these reasons, the realization of strongly directed molecular tests, aimed at the identification of genetic markers with clinical implications is recommended. In this regard, a useful genetic marker should: (I) be implicated in the tumorigenesis (such as driver alterations) because the pathway suppression could control tumor proliferation; (II) have a high prevalence, to justify the benefit of a costly test; (III) have a highly sensitive and

specific validated test; and (IV) have a previously designated oncogenic pathway, with an already available targeted therapy. Although some years ago, a panel of experts from IASLC, ATS and ERS recommended molecular testing only for the *EGFR* mutation in advanced ADC, more recent recommendations also include *EML4-ALK* rearrangement in advanced-stages of lung ADC (either locally advanced or metastatic cancer) (95). However, the latest advances in molecular profiling and guided therapies strongly suggest that the screening should already be extended to at least detection of *ROS1* fusions, *BRAF* mutations and *MET* amplifications or exon 14 alterations, performing a wider genomic profiling in any stage of ADC (26,64,96,97). This will give a more precise scenario of the phenotype epidemiology of this cancer, acting as a strong stimulus for oriented translational research (98).

The first step for the entire process is to identify the origin of the tumor using immunohistochemical techniques in the available sample. Then, the genetic profile is obtained through different techniques such as fluorescence *in situ* hybridization (FISH), polymerase chain reaction (PCR) or immunohistochemistry. For this, surgical or core-needle samples are preferred due to their larger size. However, molecular techniques can also be applied in smaller samples, such as those obtained in non-invasive or semi-invasive procedures. In this regard, multiple studies have confirmed the utility of even cytological samples obtained by endobronchial ultrasound (EBUS) to perform the molecular study (99–102). However, although a cell block can be obtained by EBUS in most cases (103), there is still controversy on its advantages and disadvantages with respect to the on-site smear in identifying driver alterations (104,105).

Genome-guided therapy

In November 2004, the first genome targeted therapy was approved by the FDA for the treatment of NSCLC with *EGFR* mutations. Since then, the prognosis of selected patients with advanced ADC and driver mutations has improved substantially. In fact, molecular testing is performed routinely in locally advanced or metastatic ADC since targeted therapies have been approved and their impact on multiple outcomes has been demonstrated. This is the case of patients with *EGFR* mutations, *EML4-ALK* rearrangement or *ROS1* fusions (64). For instance, erlenotinib, gefitinib and afatinib are used in the treatment of locally advanced or metastatic tumors with *EGFR* exon 19 deletion or exon 21 mutations, while osimertinib, olmutinib

and osimertinib are employed in the case of EGFR T790M mutations (106-112). Crizotinib, ceritinib and alectinib in turn are used in similar tumors, which in this case show *ALK* alterations. If a *ROS1* translocation is present, crizotinib can be used to treat the patients (93,113-117). More recently, promising evidence has been published with the use of crizotinib in tumors with *MET* exon 14 alterations or amplification, and dabrafenib plus trametinib in patients with *BRAF* mutations (97,118). *Table 3* summarizes the approved genome-guided therapies for lung ADC and their present indications.

Immunotherapy

Immunotherapy is a relatively novel approach for cancer, being based on the stimulation of the patient's immune system to induce a cellular-humoral response that attacks and destroys the malignant cells. Immunotherapy can be active or passive, with both being specific or non-specific. The active immunotherapy consists in the activation of the host's immune system to induce a specific response, whereas passive immunotherapy is based on the administration of antibodies that will directly kill cancer cells, without interacting with the patient's immune system. Therapy is specific if it results in a particular immune response or as general if it involves a wider immunological reaction (119).

Many scientific advances in cancer treatment are being developed in the field of active immunotherapies, whose main modalities are therapeutic vaccines and checkpoint inhibitors (120,121). The former stimulates the host immune system to generate a prolonged immunological response by recognizing tumor antigens. The vaccines can be antigen-specific or addressed to the whole-tumor, and have already been studied in the adjuvant setting, as first line and maintenance treatments, but unfortunately no positive results have been found up to now (122-124). Immune checkpoints, in turn, are inhibitory trails that control the duration and intensity of the immune response to reduce the damage in normal tissues. There are two targetable checkpoints that have been widely studied in the last years: the cytotoxic T-lymphocyte antigen 4 (CTLA-4) and the programmed death-ligand 1/programmed cell death protein 1 (PD-L1/PD-1) pathway (125).

CTLA-4 inhibitors

Two humanized monoclonal antibodies inhibiting CTLA-4 have been tested in clinical trials on patients with

NSCLC cancer. In this respect, a trial using tremelimumab in advanced-stage NSCLC showed a good tolerability profile but unfortunately showed no differences in the progression-free survival when used as a second-line agent if compared with the best supportive care (126). Two other clinical trials (ClinicalTrials.gov, numbers NCT02000947–NCT02352948), that are now in the recruitment phase, have been designed to compare dual checkpoint inhibition (anti PD-L1 and CTLA-4) using tremelimumab and durvalumab with the standard therapy (127,128).

PD-1/PD-L1 inhibitors

Under normal conditions, the PD-1 protein checkpoint protects against inflammation and autoimmunity. When a neoplasm occurs, PD-1 binds to the PD1-L1 and causes immunosuppression, preventing the immune system from attacking the tumoral cells (*Figure 3*) (129). To date, FDA has approved three PD-1/PD-L1 inhibitor drugs for the treatment of advanced-stages of NSCLC. These are nivolumab (Opdivo®, October 2015), pembrolizumab and atezolizumab (Keytruda® and Tecentriq®, respectively, both in October 2016). Nivolumab is an IgG4 monoclonal antibody that blocks PD-1 receptors expressed on activated T cells. Multiple clinical trials (CheckMate trials) have evaluated nivolumab versus docetaxel in advanced-stage NSCLC, showing an overall improved survival and a significantly better progression-free survival in the nivolumab group, with an acceptable tolerability and toxicity profile, turning this treatment into the second-line gold standard therapy in such cases (130,131). Pembrolizumab, previously called lambrolizumab, is a humanized IgG4 immunoglobulin with a high affinity for PD-1. Many clinical trials (KEYNOTE trials) have shown benefits in the overall response rate (ORR), and the overall survival in a large number of patients with advanced-stage NSCLC when compared with standard therapies, again with an excellent security profile (132,133). Ongoing studies are trying to define if pembrolizumab can be used as a first-line treatment in advanced NSCLC. Finally, a randomized, phase 3 clinical trial (OAK study), with more than a thousand patients from 31 different countries, has shown a better overall survival in patients with a previously treated NSCLC with atezolizumab when compared to docetaxel, irrespectively of PD-L1 expression (134).

In conclusion, the use of genomic phenotyping of ADC, possible now even in relatively small samples,

Table 3 Main genome-guided therapies employed with ADC and their main indications

Drug	Approved	FDA indication	EMA indication
Erlotinib (Tarceva [®])	FDA: November 2004; EMA: September 2005	First-line in metastatic NSCLC with <i>EGFR</i> exon 19 deletions or exon 21 (L858R) mutations	Treatment of locally advanced or metastatic NSCLC after failure of at least one prior chemotherapy regimen or switch maintenance treatment in stable disease
Gefitinib (Iressa [®])	FDA: July 2015; EMA: June 2009	First-line in metastatic NSCLC with <i>EGFR</i> exon 19 deletions or exon 21 L858R mutations	Treatment of locally advanced or metastatic NSCLC with activating <i>EGFR</i> mutations
Crizotinib (Xalkori [®])	FDA: August 2011; EMA: October 2012	Treatment of locally advanced or metastatic <i>ALK</i> positive NSCLC detected by a FDA-approved test	First-line and therapy of previously treated advanced <i>ALK</i> positive NSCLC
Afatinib (Giotrif [®])	FDA: July 2013; EMA: September 2013	First-line in metastatic NSCLC with <i>EGFR</i> exon 19 deletions or exon 21 (L858R) mutations detected by a FDA-approved test; second-line in advanced SqCC with disease progression after treatment with platinum-based chemotherapy	Treatment of locally advanced or metastatic NSCLC with activating <i>EGFR</i> mutations; treatment of locally advanced or metastatic NSCLC of squamous cancer progressing on or after platinum-based chemotherapy
Ceritinib (Zykadia [®])	FDA: April 2014 EMA: May 2015	Treatment of metastatic <i>ALK</i> positive NSCLC with disease progression on or that are intolerant to crizotinib	Treatment of locally advanced or metastatic <i>ALK</i> positive NSCLC
Osimertinib (Tagrisso [®])	FDA: November 2015; EMA: February 2016	Treatment of locally advanced or metastatic NSCLC with <i>EGFR</i> T790M mutations as detected by an FDA-approved test, that has progressed on or after <i>EGFR</i> tyrosine kinase inhibitor therapy	Treatment of locally advanced or metastatic NSCLC with <i>EGFR</i> T790M mutations
Alectinib (Alecensa [®])	FDA: December 2015	Treatment of <i>ALK</i> -positive metastatic NSCLC who has progressed on or is intolerant to crizotinib	–
Crizotinib (Xalkori [®])	FDA: March 2016 EMA: July 2016	Treatment of metastatic <i>ROS1</i> -positive NSCLC	Treatment of advanced <i>ROS1</i> -positive NSCLC
Olmotinib (Olita [®])	FDA: Granted breakthrough therapy designation Approved in South Korea	Treatment of locally advanced or metastatic <i>EGFR</i> T790M mutation in NSCLC	–
Dabrafenib (Mekinist [®]) + Trametinib (Tafinlar [®])	FDA: granted breakthrough therapy designation	Treatment of metastatic <i>BRAF</i> V600E-positive and previously treated NSCLC	–
Osimertinib	FDA: granted breakthrough therapy designation	Treatment of metastatic NSCLC with <i>EGFR</i> T790M mutations and TKI resistant disease	–

FDA, U.S. Food and Drug Administration; EMA, European Medicines Agency. Data obtained from the web pages of FDA (www.fda.org) and EMA (www.ema.europa.eu/ema/)

Figure 3 Main immunological events that are present in neoplasm progression.

facilitates a better tumor classification, and allows for a more targeted treatment. For this, two different strategies have been developed, genome-guided therapies, mainly based on blocking the aberrant resultant pathway, and immunotherapy, which can either be active (stimulation of the patient's immune system to produce a specific response) or passive (administration of external antibodies). Although the immune strategy is still being developed, its current results are very promising.

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Footnote

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