

Epidermal growth factor receptor and K-Ras in non-small cell lung cancer-molecular pathways involved and targeted therapies

Ramon Andrade de Mello, Dânia Sofia Marques, Rui Medeiros, António MF Araújo

Ramon Andrade de Mello, Dânia Sofia Marques, Department of Medical Oncology, Portuguese Oncology Institute, Porto 4200-072, Portugal

Ramon Andrade de Mello, 2nd Department of Medicine, Discipline of Clinical Semiotics, Faculty of Medicine, University of Porto, Porto 4200-319, Portugal

Rui Medeiros, Molecular Oncology Unit CI, IPOPGF, Porto 4200-072, Portugal

Rui Medeiros, 2nd Department of Molecular Immunology and Pathology, Instituto de Ciências Biomédicas Abel Salazar (ICBAS), University of Porto, Porto 4099-003, Portugal

António MF Araújo, Head of Lung Clinic of the Department of Medical Oncology, Portuguese Oncology Institute, Porto 4200-072, Portugal

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Correspondence to: Ramon Andrade de Mello, MD, Department of Medical Oncology, Portuguese Oncology Institute, Rua Dr António Bernardino de Almeida, S./N., Porto 4200-072, Portugal. ramonmello@med.up.pt

Telephone: +351-22-508-4000 Fax: +351-22-508-4010

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Abstract

Lung cancer is currently the leading cause of cancer death in Western nations. Non-small cell lung cancer (NSCLC) represents 80% of all lung cancers, and adenocarcinoma is the predominant histological type. Despite the intensive research carried out on this field and therapeutic advances, the overall prognosis of these patients remains unsatisfactory, with a 5-year overall survival rate of less than 15%. Nowadays, pharmacogenetics and pharmacogenomics represent the key to successful treatment. Recent studies suggest the existence of two distinct molecular pathways in the carcinogenesis of lung adenocarcinoma: one associated with smoking and activation of the *K-Ras* oncogene and the other not associated with smoking and activation of the epidermal growth factor receptor (EGFR). The *K-ras*

mutation is mainly responsible for primary resistance to new molecules which inhibit tyrosine kinase EGFR (erlotinib and gefitinib) and most of the *EGFR* mutations are responsible for increased tumor sensitivity to these drugs. This article aims to conduct a systematic review of the literature regarding the molecular pathways involving the EGFR, *K-Ras* and EGFR targeted therapies in NSCLC tumor behavior.

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Key words: Epidermal growth factor receptor; K-Ras; Non-small-cell lung carcinoma; Pharmacogenomics; p21ras proto-oncogene proteins

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INTRODUCTION

Recently, it was estimated that about 11 million people presently have cancer worldwide^[1,2]. In the United States, lung cancer (LC) is the main cause of cancer death, in both genders, and it has a global incidence of about 70 cases per 100 000 inhabitants^[3-5]. In Europe, LC incidence is about 52.5 cases per 100 000 inhabitants (82.5/100 000 in males and 23.9/100 000 in females) and mortality is approximately 48.7/100 000 (77/100 000 in males and 23.9/100 000 in females)^[3,6]. Smoking status was demonstrated in previous reports to be an important

prognostic factor due to its influence on overall survival (OS) regardless of the treatment received^[7]. Histology^[8,9], co-morbidity using the Charlson score^[10] and admission performance status^[11] also have an impact on OS and patient outcome.

Non-small cell lung cancer (NSCLC) corresponds to 80%-85% of LC and, although there is progression in the development of new chemotherapeutics, NSCLC prognosis remains unsatisfactory with a 5-year OS of less than 15%^[12,13]. Surgery is the best curative therapeutic approach in the early stages (I and II). However, even in these patients, the mean 5-year OS is less than 70%. Thus, most NSCLC patients will be candidates for adjuvant, neoadjuvant or palliative chemotherapy and/or radiotherapy at any time of the disease evolution. Although patients with metastatic disease benefit from standard chemotherapy, its impact on OS is not more than two months. This is why knowledge of the molecular pathways involved in cancer progression is very important^[1,6,14-16].

The aim of this study was to conduct a systematic review of the literature regarding the molecular pathways involving the epidermal growth factor receptor (EGFR) and *K-Ras* in NSCLC behavior and to address some issues on EGFR targeted therapies.

NSCLC PHARMACOGENOMIC CONTRIBUTION

Molecular pathway model

In the last few years, knowledge about molecular mechanisms and cellular transformation in association with cancer behavior has increased^[17-19]. More interest has been generated since the development of specific targeted therapies against the processes involved in the carcinogenesis of many types of cancers^[20-22]. During the 1990s it was discovered that the EGFR^[23] played an important role in tumoral biology and behavior^[14]. As summarized in Figure 1, EGFR stimulation activates intracellular signaling and cascades that influence cellular proliferation and mobilization, angiogenesis and other mechanisms. Normal cells are influenced by external factors, in tumor cells it was found that the activation of cell proliferation mediated by this receptor would no longer need external stimuli, but act independently and autonomously^[14,24]. In the case of NSCLC, it was shown that the over-expression of this receptor, as well as specific somatic mutations occurred in their intracellular domain with tyrosine kinase activity (between exons 18 and 21), which may influence prognosis, being significantly related to stage, survival and chemotherapy response^[14,25,26]. These data led to the development and study of various substances, including monoclonal antibodies directed to the extracellular domain of EGFR (e.g., cetuximab, Erbitux®) and small molecules that inhibit the tyrosine kinase intracellular domain (tyrosine kinase inhibitors, TKIs) of EGFR (e.g., gefitinib and erlotinib)^[14,26-30]. Preliminary results of randomized clinical trials conducted with these TKIs have shown that their use in patients with advanced

disease is effective, significantly increasing the survival of these patients, especially if they harbor mutations in the EGFR which are more frequently found in a subgroup of non-smoking, female patients, of Asian ethnicity and with adenocarcinoma histological sub-type (especially in the presence of bronchioloalveolar carcinoma). Some of these results were so impressive that this phenomenon was designated, the Lazarus effect, and led to the approval, in the United States and Europe, of erlotinib for the second- and third-line treatment of NSCLC patients; and gefitinib in Europe, for patients harboring the EGFR mutation^[25,27-29,31].

Other molecular biomarkers have been investigated in NSCLC, such as *COX-2*, *p53* and *K-Ras*^[11,32]. Among these biomarkers, *K-Ras* was shown to be important in NSCLC carcinogenesis. This biomarker is mutated in about 20% to 40% of these tumors^[26,33-35] and over 95% of the mutations described are located at codons 12 and 13, rarely at codons 59 and 61^[33]. Several environmental factors are associated with the *K-Ras* mutation, such as smoking (there is a relationship between the number of cigarettes smoked and the prevalence of mutations) and exposure to asbestos^[33,36]. Mutation of *K-Ras* appears to be an early phenomenon in NSCLC carcinogenesis and it is often associated with other molecular aberrations such as *p53* mutation, *p16* methylation, *Bcl-2*, *RASSF1* inactivation and increased expression of several growth factors, among them vascular endothelial growth factor, thereby promoting cell proliferation, suppression of apoptosis and angiogenesis^[26,33]. Although there is no consensus between the studies, *K-Ras* mutation seems to be the main poor prognostic factor in LC adenocarcinoma patients in stage I, and possibly stage II, associated with significantly lower survival rates regardless of other involved factors such as the number of treatment regimens^[26,33,35]. Similar conclusions can be drawn from the analysis of studies regarding its predictive value in response to currently recommended treatments, targeted chemotherapy and radiation therapy; furthermore, it may be a factor in resistance to therapy in the early stages (I and II), but not in advanced stages^[26,33].

Several studies have found an inverse association between *K-Ras* mutations and mutations in the EGFR tyrosine-kinase domain^[20,33]. These data suggest the possible existence of two distinct molecular pathways in lung carcinogenesis: one associated with smoking and activation of *K-Ras*; and another not associated with smoking and activation of EGFR^[28,29,36,37]. When combined, *K-Ras* mutation^[38] is mainly responsible for primary resistance to new molecules which inhibit tyrosine kinase EGFR (e.g. erlotinib and gefitinib)^[20,25,26,33,39,40].

EPIDERMAL GROWTH FACTOR RECEPTOR

C-erbB family

EGFR (or ErbB1) is a transmembrane glycoprotein encoded by a gene located on chromosome 7 (*7p12.1-12.3*).

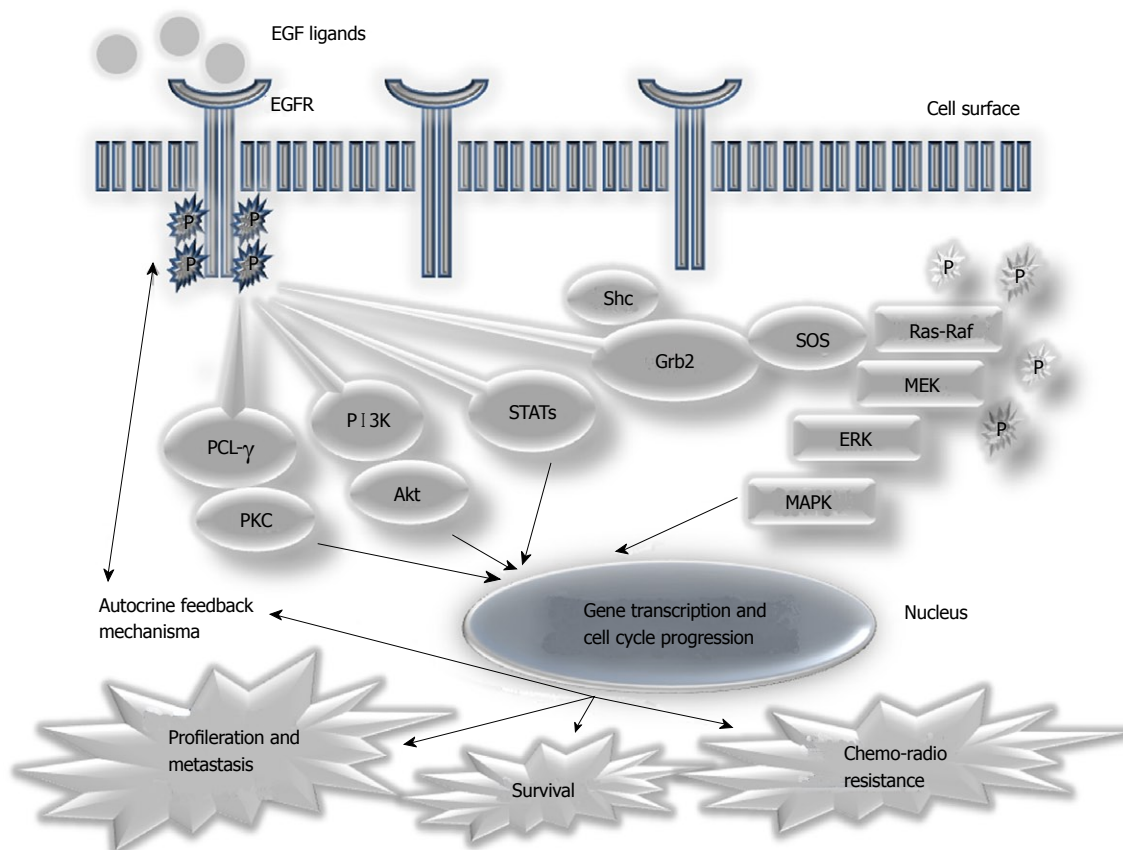


Figure 1 Molecular mechanisms through the Epidermal Growth Factor Receptor pathway. This figure shows EGFR ligands binding with their receptor and triggering mechanisms toward downstream intracellular signaling throughout the PLC γ , PI3K, Ras/Raf/MEK/ERK, STATs pathways leading to proliferation, metastasis, autocrine feedback, and survival (Adapted from reference 7 with permission). EGFR: Epidermal growth factor receptor; Shc: Src homologous and collagen protein; PLC- γ : Phospholipase C γ ; PI3K: Phosphatidylinositol 3-kinase; STATs: Signal transducer and activator of transcriptions; PKC: Protein kinase C; Grb2: Growth factor receptor bound protein 2; SOS: Guanine nucleotide exchange factor sos; MAPK: mitogen-activated protein kinase

It comprises 1186 amino acids (a.a.) and 26 exons^[41]. Exons 1-14 encode the extracellular domain, exon 15 encodes the transmembrane region and exons 16-26 the intracellular domain. This glycoprotein belongs to the ErbB receptor family, which also consists of: ErbB2 (HER2/*neu*), ErbB3 (HER3) and ErbB4 (HER4). Each of these proteins is structurally composed of an extracellular domain, a hydrophobic transmembrane domain and an intracellular domain with intrinsic tyrosine kinase (TK) activity (except ErbB3). These receptors exist as inactive monomers, being activated by their interaction, through the extracellular domain, with growth factors of the EGF family. The binding of ErbB receptor molecules to one of these ligands leads to its interaction with other monomers of the same family (receptor dimerization). This dimerization can occur between two identical receptors (homodimerization, e.g., ErbB1-ErbB1) or between two different receptors (heterodimerization, e.g., ErbB1-ErbB3). The stimulation caused by a specific ligand triggers a unique pattern of dimerization, which is also specific to the tissue/tumor in which the phenomenon occurs. Dimerization of the receptors leads to their autophosphorylation with activation of TK and activation of a cascade of intracellular biochemical processes that regulate such diverse

activities, like proliferation, differentiation, apoptosis and cell migration^[14,40] as shown in Figure 1.

Regulating the activity of tyrosine kinase

Usually, TK activity is regulated by the conformational state of the catalytic domain of the molecule. The conformation of the catalytic domain, either active or inactive, governs the ability of a kinase to transfer phosphate from adenosine triphosphate to a peptic substrate, thereby regulating the intracellular signaling pathways. There are several mechanisms that regulate this balance of active-inactive protein kinase at the atomic level. First, a.a. residues should be properly oriented in order to facilitate the transfer of phosphate and, second, the peptide substrate binding site should not be occluded. There are two important regions of the catalytic domain able to regulate these mechanisms according to their spatial orientation: the activation loop and the helix-C^[41].

In the active conformational state, the activation handle extends outside the catalytic cleft of the molecule in order to allow the substrate to bind to it, while the catalytic glutamate residue (C-helix) forms ionic interactions with a lysine residue that coordinate α and β phosphates of ATP. In the inactive conformation, the activation loop

changes its conformation drastically and hinders bonding of the peptide substrate to the catalytic domain of the molecule, while the C-helix wheel drags the residue of glutamate clear of the lysine residue^[41].

Current approaches in EGFR assessment

Mutations occurring in these TK molecule catalytic domains lead to conformational changes that promote permanent active status independent of external factors. The most common, accounting for about 85% of the mutations described, include deletions in exon 19 and substitution of a.a. leucine-858 by arginine in exon 21. These mutations increase the sensitivity to TKIs, probably by promoting conformational changes of the molecule so that the fit of the TK catalytic cleft simulates the inactive conformational status^[25,41].

On the other hand, the substitution of threonine-790 by methionine in exon 20 is a factor in resistance to TK, probably because the conformational change of the molecule caused by it does not allow the same type of effect observed for other mutations. This mutation may be acquired, having been described in patients with progressive disease after effective treatment with erlotinib and gefitinib, or innate, resulting in increased susceptibility to LC and primary resistance to molecules that inhibit TK^[25,39-42].

EGFR amplification and/or over-expression are also predictors of response to TKI treatment^[43]. The EGFR over-expression accounts for about 43%-83% of NSCLC, being more common in squamous cell carcinoma (70%), followed by adenocarcinoma (50%) and to a lesser extent, in large cell carcinoma. This phenomenon is very rare in small cell lung cancer patients^[14,39,42].

Intracellular signaling pathways

There are four main intracellular signaling pathways involved in the activation of EGFR (Figure 1): Ras/mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K)/Akt, phospholipase C γ (PLC γ), protein kinase C and signal transducer and activator of transcription (STAT)^[14,42]. Activation of PI3K leads to activation of Akt. This is translocated to the cell nucleus and mediates the transcription of many genes while other cytosolic proteins are activated simultaneously (such as mTOR and Bad), resulting in the ultimate expression of several anti-apoptotic proteins. PLC γ hydrolyzes phosphatidylinositol 4, 5-bisphosphate into diacylglycerol and inositol triphosphate with subsequent activation of protein kinase C, resulting in cell cycle progression. STAT proteins are translocated to the active nucleus and regulate transcription of genes essential for survival and proliferation, mediating cellular transformation and progression to carcinoma^[33,42]. The Ras/MAPK pathway is described in detail below.

IV-K-RAS

Ras family

The Ras family (Rat sarcoma viral oncogene), or p21ras

(so designated by the molecular weight common to the various elements that constitute the 21Kd), belongs to the super-family of small guanosine triphosphatases (GTPases) and is composed of several members (the most studied are *H-Ras*, *K-Ras* and *N-ras*)^[33,44-50].

Unlike the classic G proteins which are heterotrimeric, they remain in the form of monomeric units of connection (similar to the α subunit of classic G proteins), functioning as a small switch that toggles between the inactive monomer bound to guanosine diphosphate (GDP) and actively linked to guanosine triphosphate (GTP)^[33,44,47,49,50]. Different stimuli from the cell surface, mediated by several types of transmembrane receptors, activate these proteins, leading to a cascade of intracellular biochemical processes that regulate such diverse activities as proliferation, differentiation, apoptosis and cell migration^[33,45-50]. The most studied members of this family share high structural and functional homology (although encoded by different genes); they are expressed in all tissues (there are variations in the level of subtypes expressed); and they are implicated in the carcinogenesis of various types of tumors^[33,44-50].

Activation of K-Ras protein through the EGFR pathway

Stimulation of EGFR protein activates both *K-Ras* 4B and *H-Ras* in different ways. These are preferentially located in areas of dense cell membrane (e.g., *K-Ras* 4B), and this is the Ras protein primarily activated^[35]. The binding of EGF to its receptor causes dimerization of α and β subunits and subsequent activation of this intrinsic TK receptor, which autophosphorylates the tyrosine residues and thus allows the receptor to bind to proteins such as Shc adaptation or Grb2. These proteins serve as the link between the EGFR and a set of proteins that are able to stimulate the dissociation of guanine (GNEFs - Guanine Nucleotide Exchange Factors). GNEFs promote the dissociation of polymer Ras-GDP, releasing the Ras to bind to GTP and thus causing its activation^[33,44,46,48]. In this particular case, the first class of GNEFs to be activated is the Son of sevenless (Sos), which promotes the dissociation and activation of *K-Ras* 4B at the inner surface of the cell membrane. Secondly, through mechanisms that involve the formation of diacylglycerol, a second class of GNEFs, also called RasGRPs, promote the activation of H-Ras in the Golgi apparatus, by mechanisms not yet completely clarified^[44,46].

Generally, the Ras-GTP complex is active only transiently, since each Ras molecule has intrinsic GTPase activity that, when stimulated by GAPs, hydrolyzes rapidly to Ras-GDP binding and promotes its return to inactive baseline status and binds to GDP (GAPs increase the activity of intrinsic Ras-GTPase proteins approximately 10 000 times)^[33,44-50].

Once activated, Ras protein promotes the initiation of several distinct signaling cascades, which are reflected at the nuclear transcription of several genes and the production of factors that induce proliferation, differentiation, migration, apoptosis/cell anti-apoptosis and angio-

Table 1 Summary of studies which assessed the current main epidermal growth factor receptor targeted drugs for non small cell lung cancer

Study	Molecule	Place of study	EGFR positive selected mutations	No. of patients	Clinical stage	Response rate (%)	Median OS (mo)	Median PFS (mo)
Kris <i>et al.</i> , 2003	Gefitinib	United States	No	221	III B and IV	22	6-7	-
Perez-Soler <i>et al.</i> , 2004	Gefitinib	United States	No	57	III B and IV	12.3	8.4	-
Maemondo <i>et al.</i> , 2010	Gefitinib	Asia	Yes	230	III B and IV	73.7	30.5	10.8
Mok <i>et al.</i> , 2009	Gefitinib	Asia	No	609	III and IV	71.2	18.6	5.7
Mitsudomi <i>et al.</i> , 2010	Gefitinib	Japan	yes	177	III B and IV	62.1	30.9	9.2
Shepherd <i>et al.</i> , 2005	Erlotinib	America, Europe and Asia	No	731	III B and IV	8.9	6.7	2.2
Herbst <i>et al.</i> , 2005	Erlotinib	United States	No	526	III B and IV	30	10.6	6
Capuzzo <i>et al.</i> , 2010	Erlotinib	Italy	yes	437	III B and IV	11.9	12.3	12.3

EGFR: Epidermal growth factor receptor; OS: Overall survival; PFS: Progression-free survival.

genesis by VEGF secretion^[33,44-51]. In the case of *K-Ras* 4B, the main intracellular signaling pathways activated are the Raf/MEK/ERK (which promotes cell proliferation) and PI3K/Akt (which promotes cell survival by inhibiting apoptosis)^[33,44,46,48]. A comparison of the molecular structure of the active form (bound to GTP) and inactive form (bound to GDP) of the Ras protein showed that the transition from an active to inactive form is accompanied by changes in the conformation of the molecule into two regions, designated switch I (residues 30 to 38, the same effectors region in the center) and switch II (residues 60-76). The blockade of residues 63 to 73 using a directed antibody inhibits change of the protein binding of Ras GTP to GDP, proving that this region of the molecule is essential for conformational change of the molecule that allows the passage of the inactive and active state^[32,48,52].

As previously mentioned, Ras protein is rapidly inactivated after initial stimulation, remaining as the inactive form most of the time^[45]. However, when mutations occur, especially at codons 12, 13, 59, 61, 63, 116, 117, 119 or 146, its structure is altered by binding sites for guanine, affecting its normal function. The effects of these mutations can be translated either in a reduction of the activity of oncoprotein GTPases, blocking it into the active form bound to GTP (especially if they involve the a.a. 12, 13, 59, 61 and 63) or in decreased binding affinity and increasing the change in GDP by GTP attachment (especially if they involve the a.a. 116, 117, 119 and 146)^[35]. The inefficient deactivation of oncoprotein is intensified by the fact that GAPs have reduced ability to promote the return to the disabled state (Ras-GDP). All mutations thus facilitate accumulation of the active form (Ras-GTP), contributing to the malignant cell phenotypic change^[44,48,53].

The modified molecule is independent of stimulation by the activation of cell membrane receptors. Thus, it is understandable that patients with NSCLC who have a mutated *K-Ras* do not respond to treatment with TKI^[25]. Brugger *et al.*^[54] demonstrated that *K-Ras* works as a prognostic factor for reduced progression-free survival (PFS)

regardless of the treatment for advanced NSCLC.

The search for new targeted therapies able to inactivate *K-Ras* has led to the discovery of farnesyltransferase inhibitors^[42]. These inhibitors act at a protein level by blocking *K-Ras* farnesylation and preventing their anchor to the cell membrane. The *K-Ras* molecule, thus trapped in the cytoplasm, is not able to activate effectors of intracellular signaling pathways. Several farnesyltransferase inhibitors have been investigated in LC with unsatisfactory results. Tipifarnib, although capable of effectively blocking this enzyme, did not result in clinical response. Moreover, preliminary clinical trials conducted with lonafarbin (in combination with paclitaxel in patients with NSCLC resistant to taxanes) showed that this combination therapy was effective in controlling some 50% of patients and is currently under further Phase III clinical trials^[42].

V-CURRENT AND FUTURE ANTI-EGFR TARGETED THERAPIES

Over the few last decades, the platinum-based treatment of NSCLC has remained unsatisfactory^[55]. Many researchers have tried to identify biomarkers of response to chemotherapy such as ERCC1 (excision repair cross complementing 1) and platinum response^[56], ribonucleotide reductase subunit M1 (RRM1) and gemcitabine resistance^[57], *K-Ras* mutation and EGFR status^[38,58]. At the moment, some studies such as those shown in Table 1, have demonstrated that personalized therapy through the EGFR pathway have the potential to improve the survival of advanced NSCLC patients^[22,27,59-65]. However, the results are not linear. Many factors influence OS, PFS and response rate, such as *EGFR* mutation status, clinical TNM stage, gender and ethnicity^[66].

Erlotinib: current trends

Since 2005, based on the study by Shepherd *et al.*^[27], erlotinib has been used for second/third-line therapy^[63] to prolong survival in refractory NSCLC IIIb and IV patients irrespective of EGFR status. This drug showed

improvements in response rate, OS and 1-year-survival when added to carboplatin and paclitaxel in a recent evaluation^[67]. Furthermore, as showed in the TRIBUNE phase III trial, it was first studied in United States, Asia and Europe, but did not have consistent results following approval by the US Food and Drug Administration (FDA) and European Medicine Agency (EMA) in first-line chemotherapy patients^[27,30,61,62,68]. The genetic mutation of EGFR in exons 19 and 21 of chromosome 7 demonstrated an association with response to erlotinib^[40, 68]. Therefore, erlotinib is approved for the treatment of patients with refractory disease. In April 2010, based on the SATURN phase III trial conducted by Capuzzo *et al.*^[63], erlotinib was also approved for maintenance treatment in patients with locally advanced or metastatic NSCLC without progression after four chemotherapy cycles in the first-line setting. In 2011, an American Society of Clinical Oncology provisional opinion panel started to consider erlotinib as first-line therapy for advanced NSCLC^[69]. A recent Chinese phase 3 trial (OPTIMAL, CTONG 0802), enrolled 83 patients with advanced NSCLC and mutations of the EGFR gene (exon 19 and 21) from 22 centers in China^[70]. This study was conducted in order to compare the efficacy and tolerability of the TKI erlotinib *versus* standard chemotherapy, in this case gemcitabine plus carboplatin. They showed significantly higher PFS in patients treated with erlotinib compared with those treated with standard chemotherapy: 13.1 mo (95% CI 10.58-16.53) *vs* 4.6 months (95% CI 4.21-5.42). Furthermore, grade III and IV toxicities, mainly neutropenia and thrombocytopenia, were more frequent in the chemotherapy arm. Thus, these findings suggested that erlotinib might be an important agent in the first-line treatment of advanced NSCLC patients with positive EGFR mutations (mainly in exon 21). Based on these results, erlotinib was approved in Europe in September 2011, as first-line therapy in patients with locally advanced or metastatic NSCLC harboring EGFR activating mutations. Currently, erlotinib is also recommended as second- and third-line therapy in a subset of advanced NSCLC patients irrespective of their EGFR status, due to its impressive results described in the above studies^[27,63,70].

Gefitinib

Gefitinib did not initially show significant clinical benefit on OS, PSF and tumor response in Western patients with NSCLC IIB and IV stages^[59,61]. The Iressa survival evaluation in lung cancer (ISEL) trial showed disappointing results when no improvement in OS was observed in patients treated with gefitinib in either the overall or adenocarcinoma population^[60]. However, others studies (IPASS) reported the superiority of gefitinib when compared with platinum-taxane-based therapy protocols mainly in patients with EGFR mutations, adenocarcinoma histology, nonsmokers or former light smokers in East Asia^[64,65]. Subsequently, it was also confirmed that EGFR mutations had a predictive role in the response of lung ad-

enocarcinoma to gefitinib as compared with carboplatin-paclitaxel treatment^[43,64,70,71]. These findings resulted in the approval of gefitinib by EMEA for use in the first-line treatment of patients with advanced metastatic NSCLC EGFR mutation positive tumors^[65].

The role of MET targeted inhibition

Recently, the MET proto-oncogene was discovered which encodes for the high affinity cell surface receptor for hepatocyte growth factor (HGF) and also control the main steps of carcinogenesis: cell growth, invasion, proliferation and apoptosis^[72,74]. Thus, MET inhibitors emerged as a promising new class of targeted drugs in patients with MET-mediated resistance to EGFR inhibitors^[66,74]. Nowadays, dual MET-EGFR multi-target TKI therapies may be considered a good approach for MET-mediated resistance to EGFR inhibitors to improve NSCLC patient outcome. In 2010, a recent multi-target of MET, VEGFR2 and RET, called XL 184, in association with erlotinib in NSCLC EGFR T790M and MET amplified patients was presented at the American Society of Clinical Oncology annual meeting as a promising choice in these patients^[75]. Another drug, ARQ197, which is a selective non ATP competitive inhibitor of c-MET, when combined with erlotinib in the second/third-line treatment of EGFR inhibition *naïve* NSCLC patients showed increased PSF, mainly among patients with non-squamous histology, K-Ras mutations, and EGFR wild-type status^[73]. Other drugs targeting MET pathways, such as AMG102^[74], a monoclonal antibody against HGF, and MetMab (Genentech)^[77], a human recombinant agonist of the HGF-Met signaling pathway, are still in phase I studies and show promise in the treatment of NSCLC patients^[76-78].

EML4-ALK mutation and the role of crizotinib in EGFR TKI resistant patients

In the last few years, the echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase (EML4-ALK) fusion gene has been identified by Fluorescence in Situ Hybridization (FISH) Assay as an oncogene in about 11.3% of patients with NSCLC^[79]. These patients are resistant to EGFR TKI therapies and should be directed to ALK-targeted agents^[80]. FISH and real time polymerase chain reaction represent two primary methods to assess ALK fusions^[81]. Recently, crizotinib, was identified as a potent inhibitor of ALK and MET tyrosine kinases^[82,83]. It was demonstrated in previous studies that this drug was well tolerated and resulted in important tumor shrinkage in NSCLC EML-4ALK positive patients^[84, 85]. Crizotinib has been shown to significantly control disease when used in NSCLC patients with EML4-ALK mutation fusion who were refractory to EGFR TKI treatment^[82,86]. However, some patients have resistance to crizotinib and other EML4-ALK inhibitors are in development^[87]. Other studies^[88] showed that EML4-ALK mutation prolonged PSF in patients treated with premetrexed. Thus, this should be considered in trials involving patients treated with this drug. Recently, it was reported

that crizotinib does not cross the blood brain barrier and thus its cerebrospinal fluid levels are insufficient to control brain metastases^[89]. Further studies are warranted to assess this situation.

CONCLUSION

In recent decades, therapeutic advances in LC studies, with the use of combined platinum-based chemotherapy strategies, radiotherapy or surgery, have not been completely satisfactory in terms of overall survival; and thus the prognosis associated with this disease remains very poor^[54,90-95]. The need to find new targeted agents has renewed interest in the study and understanding of the molecular pathways involved in lung carcinogenesis^[3,9,17,18,58,91,92], and several targeted therapeutic molecules have been synthesized^[22,96-99]. However, it has also become evident that there are multiple pathogenic mechanisms in lung cancer working in parallel or with several loops of activation/inhibition, thus therapeutic exploration with the goal of disease control can not be based on the study of a single mechanism^[22,97-99]. Through experience with molecules such as gefitinib and erlotinib, it is now understood that the benefit of EGFR TKIs depend on several biological characteristics in individual patients^[30,59,100-103]. The study of new targeted agents and their combination in order to optimize therapy should therefore take into account the individual characteristics of each patient^[55,58]. This is currently a promising field of cancer research in which genetics, tumor molecular biology and clinical experience interact to achieve more effective combination therapies adjusted to the patient profile.

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