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Review

# Pharmacogenomics and targeted therapy of cancer: Focusing on non-small cell lung cancer

Seyyed Mortaza Haghgoo [a], Abdolamir Allameh [a], Esmaeil Mortaz [b,c,d,e,*], Johan Garssen [e,f], Gert Folkerts [e], Peter J. Barnes [b], Ian M. Adcock [b]

[a] Department of Clinical Biochemistry, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[b] Airways Disease Section, National Heart and Lung Institute, Imperial College London, London, UK
[c] Department of Immunology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[d] Chronic Respiratory Diseases Research Center and National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Masih Daneshvari Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[e] Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht, The Netherlands
[f] Nutricia Research, Immunology, Utrecht, The Netherlands

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ABSTRACT

Recent studies have been established high degree of genetic diversity in solid organ tumors among individuals and even between individual tumor cells. This intratumor and intertumor genetic diversity results in a heterogeneous tumor with unique characteristics which potentially allows effective drug therapy. The goal of pharmacogenomics is to elucidate the genetic network(s) that underlie drug efficacy and drug resistance. Advances in targeted and personalized therapy play an increasingly important role in many common cancers, notably lung cancer, due to the high incidence, prevalence, mortality and the greater tendency towards drug resistance seen in these patients. Non-small cell lung cancer (NSCLC) is characterized by mutations in the epidermal growth factor receptor (EGFR) and or downstream kinase pathways. This has led to the development of highly selective monoclonal antibodies and EGFR tyrosine kinase inhibitors (EGFR-TKIs) to prevent cancer initiation, proliferation, differentiation, angiogenesis, survival, and invasion. However, resistance to many of these new treatments is induced and further pharmacogenomic analysis has revealed mutations associated with increased or reduced drug efficacy. Combinations of kinase inhibitors or potentially the targeting of cancer stem cells may further increase the success of pharmacogenomics in treating patients with lung cancer.



**Contents**



*Abbreviations:* ALDH, aldehyde dehydrogenase; ALK, anaplastic lymphoma kinase; BRAF, v-Raf murine sarcoma viral oncogene homolog B1; CSC, cancer stem cell; EGFR, epidermal growth factor receptor; EGFR-TKIs, EGFR tyrosine kinase inhibitors; EGFRvIII, EGFR variant III; EML4, echinoderm microtubule-associated protein-like 4; FISH, fluorescence in situ hybridization; FDA, America Food and Drug Administration; G6PD, glucose-6-phosphate dehydrogenase; HGFR, hepatocyte growth factor receptor; IACR, International Agency for Research on Cancer; IHC, immunohistochemistry; JAK/STAT, janus kinase/signal transducer and activator of transcription; K-RAS, V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; LOH, loss of heterozygosity; MAPKs, mitogen-activated protein kinases; NSCLC, non-small cell lung cancer; NTRK1, neurotrophic tyrosine kinase receptor type 1; PI3K/AKT, phosphatidylinositide 3-kinases/protein kinase B; RET, rearranged during transfection; ROS1, c-ros oncogene 1; RTK, receptor tyrosine kinase; RT-PCR, reverse transcription polymerase chain reaction; SCC, squamous cell carcinoma; SCLC, small cell lung cancer; SNPs, single nucleotide polymorphisms; VEGF, vascular endothelial growth factor

* Corresponding author at: Airways Disease Section, National Heart and Lung Institute, Imperial College London, London, UK.
*E-mail address:* e.mortaz@uu.nl (E. Mortaz).

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## 1. Introduction

The term of *genetic* was first used by William Bateson in 1900 to describe his studies of human Mendelian inheritance (Charlab and Zhang, 2013; Meyer, 2004). Two years later, Archibald Grove and colleagues observed that diseases such as Pentosuria and Alkaptonuria were inherited in an autosomal recessive manner and introduced the concept of *chemical individuality* for the first time (Charlab and Zhang, 2013; Meyer, 2004). Nevertheless, the beginning of pharmacogenetics is attributed to Laurence Snyder's study on "Inheritance of phenylthiocarbamide taste recognition" (Meyer, 2004). He indicated that only some people were able to taste *phenylthiocarbamide* and that this ability was inherited in an autosomal recessive manner (Meyer, 2004). As such, Snyder demonstrated the relationship between inheritance and the response to an intervention.

The clinical importance of inheritance and the response to treatment was demonstrated in the 1950s with the appreciation of the relationship between a glucose-6-phosphate dehydrogenase (G6PD) defect and the occurrence of hemolysis during treatment with primaquine (an antimalarial drug) (Carson et al., 1956; Clayman et al., 1952; Meyer and Zanger, 1997). Simultaneously, a link between the response to isoniazid (an anti-tuberculosis drug) and an autosomal recessive defect in enzymatic acetylation was established (Blum et al., 1991; Evans et al., 1960; Hughes et al., 1954; Vatsis et al., 1991). Also, at this time, genetic defects in other drug metabolizing enzymes were realized as being important in the cause of death in affected individuals (Meyer, 2000, 2004; Wood et al., 2003). Friedrich Vogel and colleagues applied the term "pharmacogenetics" to such studies. The term "Pharmacogenomics" was introduced in 1989 to encompass the involvement of complex genetic networks behind drug resistance, efficacy, and side effects (Charlab and Zhang, 2013; Meyer, 2004; Nebert et al., 2008; Wang et al., 2011; Weinshilboum and Wang, 2006; Whirl-Carrillo et al., 2012).

In has become clear that cancers, particularly solid organ tumors, have a high degree of genetic diversity (Balmain et al., 2003; Morin et al., 2008). Indeed, solid tumors may have up to 100 mutated genes which vary between individual cells within the tumor and as a result it is often unclear what the driver mutations are. The realization that many driver mutations are linked to a smaller number of pathways which are critical for oncogenesis has highlighted the need for tumor analysis at the molecular level. This approach has increased our understanding of the basis for NSCLC pharmacogenomics.

Targeted therapy is a powerful strategy for cancer treatment and overcome drug resistance (Gerber, 2008). The accumulation of knowledge about the differences between normal and cancer cells and differences among cancer cells has allowed for the development of new anticancer agents which target key molecules involved in cancer initiation, proliferation, differentiation, angiogenesis, survival, and invasion. (Gerber, 2008; Hanahan and Weinberg, 2011; Luo et al., 2009). In this review we summarized the history of pharmacogenomics and its potential in personalized medicine. We also discuss the place of pharmacogenomics in cancer-targeted therapy with a focus on NSCLC.

## 2. Pharmacogenomics and cancer therapy

Cancer progression is related to the combined effects of functional changes in cell membrane receptors and intracellular signaling pathways which modulate cell proliferation, apoptosis, motility, adhesion, and angiogenesis (Hanahan and Weinberg, 2011). Human cancer genome sequencing has detected a series of genetic changes that occur in different cancers. Single nucleotide polymorphisms (SNPs), haplotypes, microsatellites, insertion or deletion of nucleotides (Ins/Del), copy number variations, aneuploidy, and loss of heterozygosity (LOH) are the most common genetic changes reported to be associated with uncontrolled growth and metastasis (Pleasance et al., 2010; Savonarola et al., 2012; Stratton et al., 2009). In addition, the resistance of cancer cells to various drugs has been associated with a number of processes including the increased expression of cell membrane transporter proteins, changes in the activity of cellular proteins involved in detoxification, DNA repairing, apoptosis and activation of oncogenes/inactivation of tumor suppressor proteins (Luo et al., 2009).

The high prevalence of drug resistance in NSCLC, especially in advanced stages of disease, has driven the increase in the number of pharmacogenomics studies (Gadgeel et al., 2010; Pao et al., 2005a; Stewart et al., 2004; Tsuruo, 2003). Indeed, the American Food and Drug Administration (FDA) strongly recommends pharmacogenomics testing before the prescription of several anticancer agents in order to avoid, or at least minimize, possible life-threatening side effects and to reduce the costs of ineffective treatment (Coleman, 2014; Meyer, 2000).

## 3. Non-small cell lung cancer

Lung cancer is the leading cause of cancer-related death worldwide (Jemal et al., 2011; McErlean and Ginsberg, 2011). The International Agency for Research on Cancer (IACR) has estimated that the number of deaths due to lung cancer will increase to ten million deaths per year by 2030 (Minna and Schiller, 2008). The main risk factor for lung cancer is smoking and 75–90% of patients have a history of smoking (McErlean and Ginsberg, 2011). The term lung cancer usually refers to tumors that originate from the lining cells of the respiratory tract (epithelial cells) (Minna and Schiller, 2008). Based on differences in biological characteristics, lung cancer is classified into two types, namely non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC accounts for approximately 85% of lung cancer cases (Minna and Schiller, 2008; Reck et al., 2013). Platinum-based chemotherapy is prescribed as the standard first-line therapy in patients with advanced NSCLC (Minna and Schiller, 2008). However, resistance to platinum-based drugs reduces the survival rate which, as a

result, has not improved to anything like the extent seen in other cancers. Recent data, however, suggests that targeted therapy linked to analysis of predictive biomarkers may be one way to overcome drug resistance. Considering the prominent role of receptor tyrosine kinases (RTK) such as epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK) in NSCLC pathogenesis and progression it is no wonder that the approval of a number of small molecule tyrosine kinase inhibitors represented a landmark in the treatment of NSCLC and a significant step towards the goal of personalized medicine in lung oncology (Gerber, 2008; Hanahan, 2014; Roskoski, 2014).

### 3.1. EGFR mutations

The EGFR signaling pathway has become recognized as a key pathway implicated in NSCLC (Sharma and Settleman, 2009). EGFR is a member of the ErbB family of RTK (Sharma and Settleman, 2009). Ligand binding to EGFR induces receptor dimerization and autophosphorylation of its intracellular domain which subsequently leads to activation of downstream signaling cascades such as the mitogen-activated protein kinases (MAPKs), the phosphatidylinositide 3-kinase/protein kinase B (PI3K/AKT) and the janus kinase/signal transducer and activator of transcription (JAK/STAT) pathways resulting in increased cell proliferation and survival (Fig. 1) (Schneider and Wolf, 2009; Tomas et al., 2013). The EGFR gene is located on the short arm of chromosome 7 (7p12) and the kinase domain is encoded by exons 18–24 (Fig. 2) (Sharma and Settleman, 2009). To date, dozens of mutations in the EGFR kinase domain have been described which affect the response to treatment with EGFR tyrosine kinase inhibitors (EGFR-TKIs) (Sharma et al., 2007b).

Mutations usually occur in exons 18–21 and cause either resistance or sensitivity to EGFR-TKIs (Fig. 2). Mutations in exons 18, 19, and 21 are predominantly associated with enhanced sensitivity to EGFR-TKIs whilst mutations in exon 20 e.g. T790M are mainly associated with resistance to these drugs (Sharma et al., 2007b). The first generation EGFR-TKIs such as Erlotinib (Tarceva®) and Gefitinib (Iressa®) compete with ATP for binding at the tyrosine kinase domain (Ward et al., 1994) and are licensed for use in patients with tumors positive for drug susceptibility mutations. Mutations associated with EGFR-TKI resistance are rare before the onset of EGFR-TKI treatment and generally occur after the start of treatment. The T790M mutation is identified in 50% of patients who acquire resistance to EGFR-TKIs (Linardou et al., 2009; Pao et al., 2005a; Shigematsu and Gazdar, 2006). This acquired resistance to EGFR-TKIs is common in cancer and highlights the ability of tumors to adapt to pathway inhibition using a variety of processes to bypass the blockade. The irreversible EGFR-TKI Afatinib (Gilotrif®, previously Tomtovok and Tovok) is effective against cancers resistant to Erlotinib and Gefitinib and has been shown also possesses Her2 inhibtiory activity. The recent Lux-Lung clinical trial program has demonstrated the effectiveness of Afatinib in patients with advanced NSCLC (Metro and Crinò, 2011; Rossi and Di Maio, 2015).

Studies conducted 10–15 years ago indicated the presence of two mutations in exons 19 and 21 of the EGFR gene, deletion (ΔE746-A750) and point mutations (L858R) (Lynch et al., 2004; Paez et al., 2004) that are common and which can predict the response to treatment with EGFR-TKIs in patients with NSCLC. These mutations are observed in 10–40% of cases of NSCLC (Sharma et al., 2007a). Third generation irreversible EGFR-TKIs such as CO-1686, WZ-4002, and AZD-9291 have been developed which act as mutant-selective agents against both T790M and the initial EGFR mutations but not against wild-type EGFR. These drugs may be used as either monotherapies or as components of combination therapy (Heuckmann et al., 2012; Pao and Chmielecki, 2010; Zhou et al., 2009).

Mutations associated with sensitivity to EGFR-TKIs cause structural changes at the protein level and reduce the affinity of ATP for the active site of the kinase thereby augmenting TKI sensitivity (Pao and Chmielecki, 2010). “Oncogene addiction”, proposed by Bernard Weinstein in 2000, has also been proposed as the reason for enhanced susceptibility to EGFR-TKIs. Oncogene addiction refers to the phenomenon by which a cancer cell, despite many other genetic changes, can become completely depend on one oncogenic pathway for its proliferation and survival. Determination of the EGFR mutations present in an individual cancer allows clinicians to better predict the response to treatment with EGFR-TKIs (Weinstein, 2000, 2002; Weinstein and Joe, 2006).

The standard method for the detection of these mutations is direct sequencing. Recently, two FDA-approved PCR-based companion diagnostic assays (Cobas by Roche and Therascreen by Qiagen) have been validated for use on formalin fixed paraffin embedded (FFPE) tissue (Food and Drug Administration, 2013a, 2013c). These take advantage of the development of highly selective monoclonal antibodies that can detect mutations associated with EGFR-TKI sensitivity and may be used in a primary screening test to assess potential treatment responses. However, there are some limitations to these assays in that not all mutations are detected and there is no well-defined cut-off point to determine the presence or absence of mutations (Brevet et al., 2010; Yu et al., 2009).

#### 3.1.1. EGFR variant III

EGFR variant III (EGFRvIII) results in deletion of exons 2–7 in EGFR gene which results in the loss of 268 amino acids (aa 6 to 273). This mutation is commonly associated with squamous cell carcinoma (SCC). Deletion of exons 2–7 results in a protein product with profound functional changes including the loss of the ability to bind to EGF and a constant activation of the tyrosine kinase domain. EGFRvIII has also been reported in other cancers such as glioblastoma, breast cancer, ovarian cancer and prostate cancer. The clinical importance and incidence of EGFRvIII is not clear although studies indicate that the presence of EGFRvIII is associated with resistance to Erlotinib and Gefitinib but does not affect the susceptibility to irreversible EGFR-TKIs such as Afatinib (Ji et al., 2006; Ohtsuka et al., 2007; Sasaki et al., 2007).

### 3.2. K-Ras mutations

K-Ras (v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog) is a membrane-bound oncoprotein and a member of the Ras protein superfamily. The *K-Ras* gene is located on the short arm of chromosome 12 (12p12.1) and its protein product plays an important role in several cell signaling pathways including activation of the MAPK and PI3K/AKT pathways. K-Ras is a GTPase and binding of GTP to the active site acts as a molecular on/off switch. Normally K-Ras binds GTP and hydrolyzes it to GDP and phosphate. K-Ras is turned off upon conversion of GTP to GDP leading to regulation of cell proliferation and growth (Mitin et al., 2005; Wennerberg et al., 2005). Mutations in the *K-Ras* gene can result in GTP being permanently bound to K-Ras leading to the uncontrolled proliferation and growth of tumor cells (Macaluso et al., 2002; Shigematsu and Gazdar, 2006).

Approximately 25–35% of NSCLC patients are diagnosed with activating mutations in K-Ras. K-Ras mutations are more common in patients with adenocarcinoma, who have a history of smoking, than in any other subtype of NSCLC. The most common mutations occur in codons 12 and 13 of exon 2 (Jang et al., 2009; Riely et al., 2009). Studies have shown that K-Ras mutations can also be associated with resistance to EGFR-TKIs (Fig. 3) (Linardou et al.,

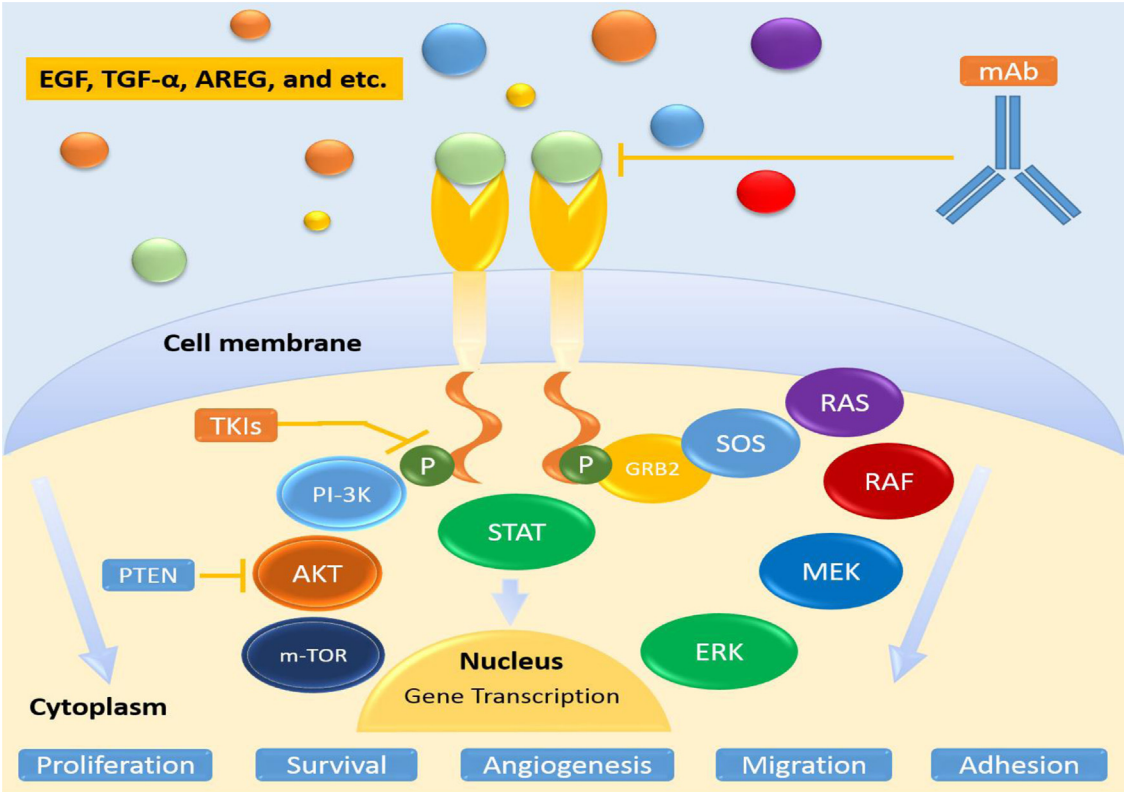


**Fig. 1.** The epidermal growth factor (EGF) signaling pathway. Binding of ligands such as EGF, transforming growth factor alpha (TGF-α), and amphiregulin (AREG) to the EGF receptor (EGFR) leads to activation of several key downstream signaling pathways including the phosphatidylinositide 3-kinases/protein kinase B (PI3K/AKT), Signal Transducer and Activator of Transcription (STAT) and the mitogen-activated protein kinase (MAPK) cascades. The MAPK pathway consists of the extracellular regulated kinase (ERK) and its upstream amplifying kinases MEK, RAF, RAS, SOS and GRB2. Mammalian target of rapamycin (m-TOR) is the downstream target of AKT which is regulated by the tumour suppressor phosphatase and tensin homolog (PTEN). Activation of these pathways leads to cell proliferation, survival, adhesion, migration and angiogenesis. Drugs including monoclonal antibodies (mab) such as Cetuximab can bind to extracellular region of EGFR to block receptor activation whilst others known as EGFR tyrosine kinase inhibitors (EGFR-TKIs) target the intracellular tyrosine kinase domain to prevent receptor activation.

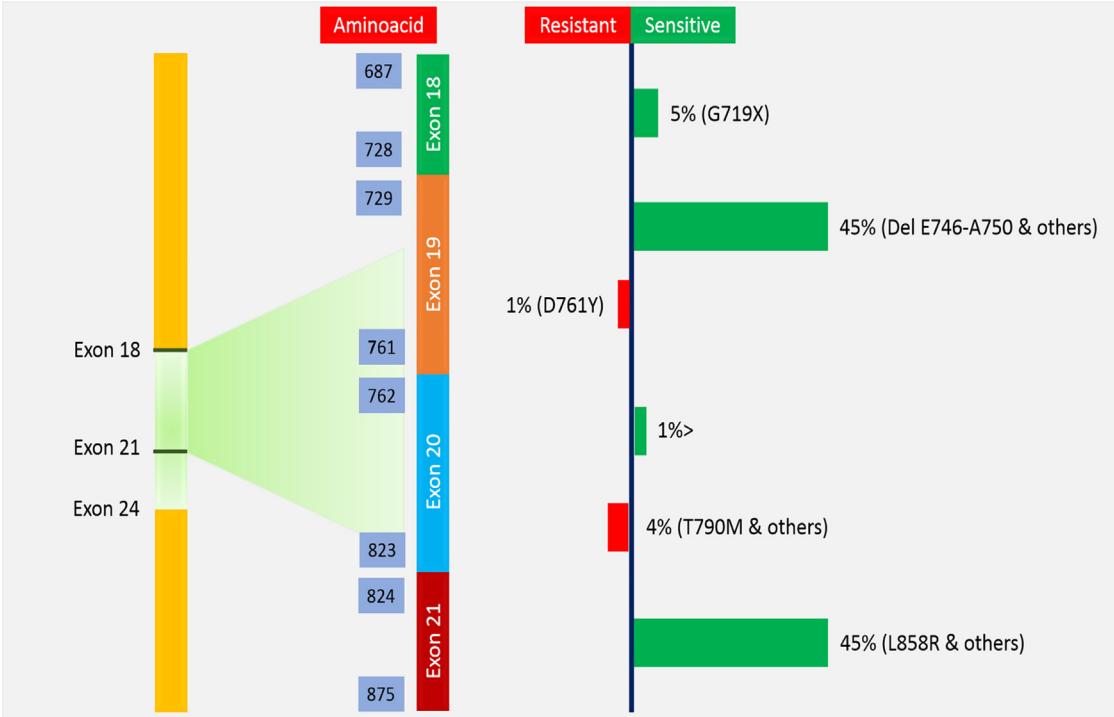


**Fig. 2.** Exons 18–24 encode the tyrosine kinase domain of epidermal growth factor receptor (EGFR). Mutations which affect the resistance and susceptibility to EGFR tyrosine kinase inhibitors (EGFR-TKIs) occur in exons 18–21. Mutations that increase susceptibility to EGFR-TKIs are commonly located on exons 18, 19 and 21 whilst mutations associated with resistance to EGFR-TKIs are generally located in exon 20. Point mutations in exon 18 causing the substitution of a Leucine for Arginine (L858R) (45%) or the deletion of amino acids 746–750 due to deletion mutation in exon 21 (45%) are most frequently observed in patients with NSCLC.

2008; Massarelli et al., 2007; Pao et al., 2005b). However, generally EGFR and K-Ras mutations are mutually exclusive and resistance to first generation EGFR-TKIs may be due to the presence of wild type EGFR rather than the presence of K-Ras mutations (Schmid et al., 2009). K-Ras mutations are also associated with other problems such as thrombosis in cancer patients and these mutations have been used as a prognostic biomarker for NSCLC in a number of studies (Marks et al., 2008; Young et al., 2012). Although K-Ras appears to be a logical druggable target, no drug has been developed and approved for inhibition of mutant K-Ras and alternative strategies such as inhibition of heat shock protein 90 have been suggested as treatments for patients with these mutations (Banerji, 2009; Vakiani and Solit, 2011).

### 3.3. EML4–ALK rearrangement

Anaplastic lymphoma kinase (ALK) is another tyrosine kinase receptor and its gene is located on the short arm of chromosome 2 (2p23) (Fig. 4) (Chiarle et al., 2008). Fusion of the ALK gene with the echinoderm microtubule-associated protein-like 4 (EML4) gene, located on short arm of chromosome 2 (2p21), is observed in 5% of NSCLC cases (Chiarle et al., 2008). The first report of an EML4–ALK fusion gene in NSCLC was published in 2007 (Soda et al., 2007). The EML4–ALK fusion gene results in a protein with persistent ALK kinase activity and uncontrolled cell growth, proliferation and survival (Chiarle et al., 2008; Hallberg and Palmer, 2013; Sasaki et al., 2010; Soda et al., 2007) (Fig. 4). The gold standard method for detection of EML4–ALK rearrangement is FISH (fluorescence in situ hybridization) (Martelli et al., 2009; Shaw et al., 2009) and a diagnostic test, the Vysis break apart FISH assay, has got pre-marketing approval from the FDA for use in FFPE tissue (Food and Drug Administration (FaD), 2013).

Crizotinib (Xalkori®) was approved for the treatment of ALK positive NSCLC patients in 2013 (Food and Drug Administration, 2013b; Kwak et al., 2010; Roberts, 2013; Sahu et al., 2013) and is significantly more effective than chemotherapy in previously untreated patients (Solomon et al., 2014). However, a minority of NSCLC patients who are EML4–ALK positive do not response to Crizotinib and patients who initially respond to Crizotinib often only have a transient response. The reasons for the lack of response and the drug-acquired failure are unknown. Mutations such as C1156Y and L1196M can occur in the ALK kinase domain but no link to drug responsiveness has been reported (Camidge and Doebele, 2012; Choi et al., 2010; Lovly and Carbone, 2011; Mok, 2011). Ceritinib (Zykadia®) (an ALK inhibitor) was approved by the FDA in April 2014 for the treatment of patients with ALK positive, metastatic NSCLC with disease progression or for those who are intolerant to Crizotinib (Food and Drug Administration, 2014). It is noteworthy that initial studies with second generation ALK tyrosine kinase inhibitors including LDK-378 and AP-26113 have shown high response rates in patients with acquired Crizotinib resistance (Gridelli et al., 2014; Perez et al., 2014).

### 3.4. BRAF mutation

Activating mutations in the BRAF (v-Raf murine sarcoma viral oncogene homolog B1) gene that increase its kinase activity have also been associated with resistance to EGFR-TKIs in NSCLC. BRAF is a serine/threonine kinase downstream of K-Ras signaling (Fig. 3) (Davies et al., 2002; Wan et al., 2004). BRAF mutations usually occur with low incidence in adenocarcinomas (Paik et al., 2011). In 50% of cases, a mutation in exon 15 leads to the substitution of valine for glutamic acid at position 600 in BRAF (V600E). Substitutions of glutamine for alanine due to a mutation in exon 11 and substitution of aspartate for glutamine due to a mutation in exon 15 are observed in 39% and 11% of cases, respectively (Brose et al., 2002). The majority of clinical trials to date have focused on

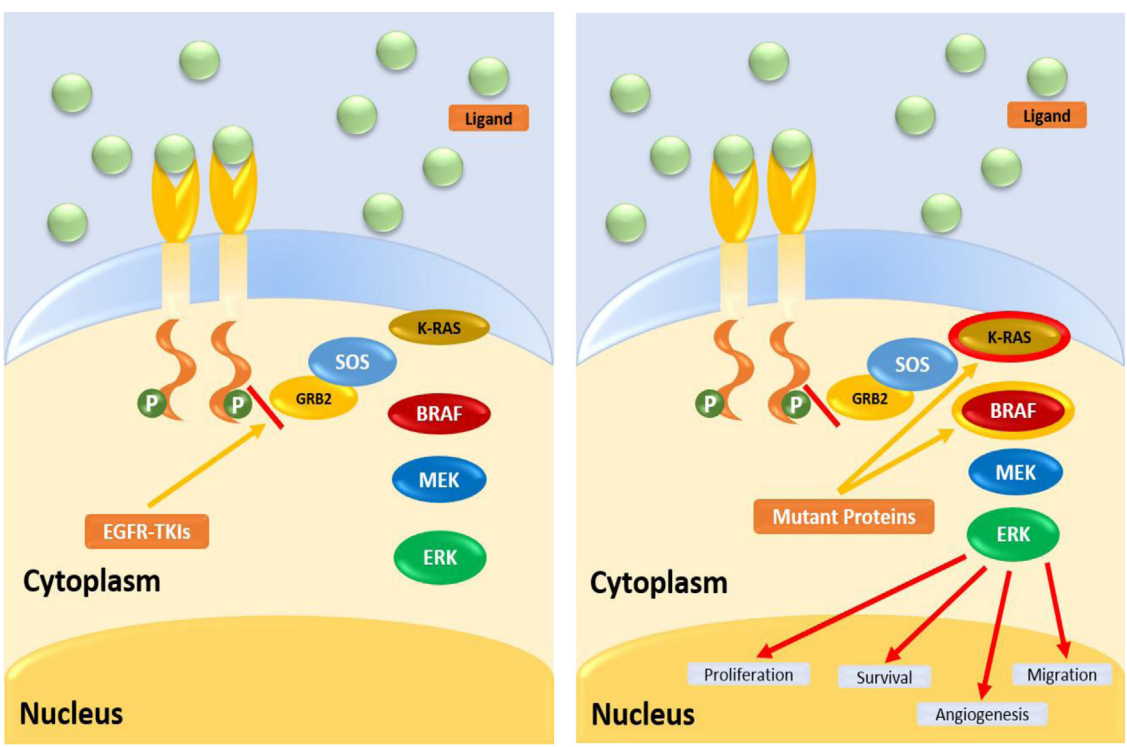


**Fig. 3.** Mutations downstream of the epidermal growth factor receptor (EGFR) the efficacy of EGFR tyrosine kinase inhibitors (EGFR-TKIs). Left panel: EGFR-TKIs bind to the EGFR intracellular region and prevent activation of downstream mitogen-activated protein kinase (MAPK) cascades. The MAPK pathway consists of the extracellular regulated kinase (ERK) and its upstream amplifying kinases MEK, BRAF, K-RAS, SOS and GRB2. Right panel: Mutations in downstream kinases such as K-Ras and BRAF lead to persistent activation of the MAPK pathway leading to resistance to EGFR-TKIs and cell proliferation, survival, migration and angiogenesis.

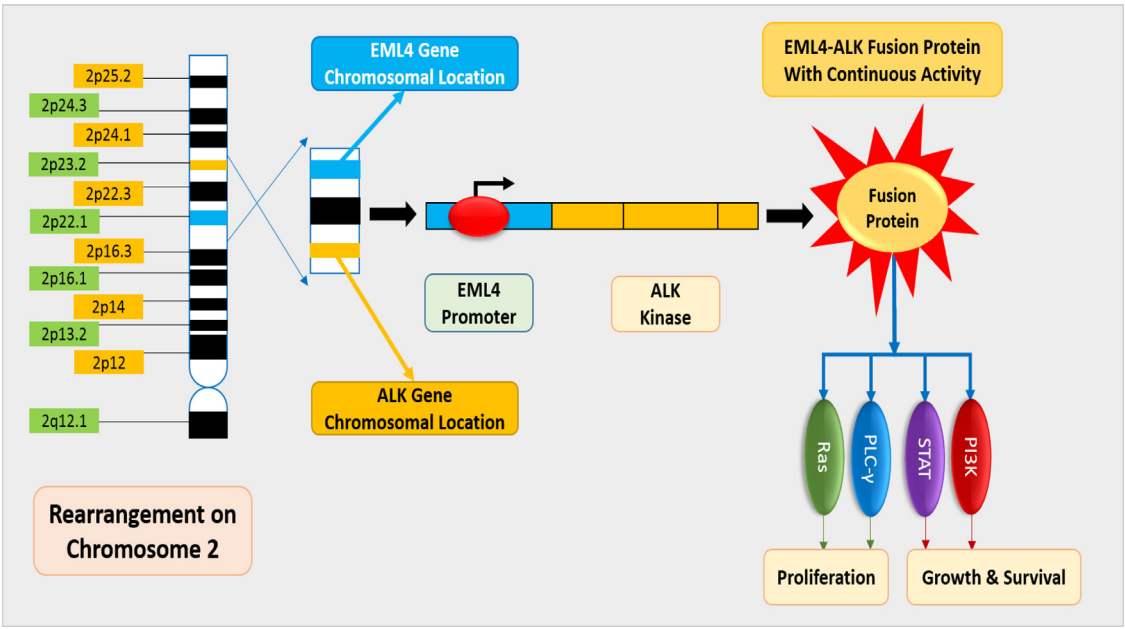


**Fig. 4.** Rearrangement of the short arm of chromosome 2 leads to formation of an echinoderm microtubule-associated protein-like 4 (EML-4)-anaplastic lymphoma kinase (ALK) (EML-4-ALK) fusion protein. The EML-4-ALK fusion protein has persistent kinase activity causing the enhanced activation of downstream pathways including the mitogen activated protein kinase (MAPK), phospholipase C (PLC)-γ, signal transducer and activator of transcription (STAT) and phosphatidylinositide 3-kinase (PI3K) pathways. The pathways regulate cell proliferation, growth and survival.

melanoma since >90% of melanoma cases have the V600E mutation (Davies et al., 2002; Paik et al., 2011).

The FDA has approved BRAF-specific inhibitors such as Vemurafenib (Zelboraf®) and Dabrafenib (Tafinlar®) for the treatment of metastatic melanoma (Huang et al., 2013). These drugs show clinical efficacy with acceptable safety profiles although drug-acquired loss of efficacy does occur. This loss of efficacy may be prevented using combination therapy with drugs such as the MEK inhibitor Trametinib (Mekinist®) (Flaherty et al., 2012). Clinical trials in NSCLC patients enriched for BRAF mutations are needed.

### 3.5. MET amplification

MET also called hepatocyte growth factor receptor (HGFR) is another cell surface tyrosine kinase receptor (Maulik et al., 2002). When oncogene addiction occurs in tumor cells, inhibition of EGFR downstream proteins can result in a "kinase switch" to ensure cell survival. One of the main switch pathways activated is the tyrosine kinase MET (Maulik et al., 2002; Tulasne et al., 2004). Increased expression of MET leads to acquired resistance to the EGFR-TKIs and ~20% of EGFR-TKI-resistant patients have increased MET amplification (Brugger and Thomas, 2012). MET inhibitors such as Cabozatinib (Cometriq®, formerly known as XL184) has been approved by the FDA for the treatment of some cancers, but no clinical data is yet available on the efficacy of Cabozatinib in patients with NSCLC (Christensen et al., 2005; Food and Drug Administration, 2012).

### 3.6. Rare genetic alterations in NSCLC

Recently, several genetic alterations have been discovered in NSCLC which have clinical importance in targeted therapy. C-ros oncogene 1 (ROS1) and rearranged during transfection (RET) are tyrosine kinase receptors and associated genes located on chromosomes 6 (6q22) and 10 (10q11.2), respectively (Birchmeier et al., 1986; Gainor and Shaw, 2013; Nagarajan et al., 1986; Takahashi et al., 1985). ROS1 and RET rearrangements result in formation of fusion kinases capable of oncogenic transformation (Birchmeier et al., 1987; Grieco et al., 1990). These rearrangements seldom occur simultaneously with other genetic alterations such as those described above for EGFR, K-Ras, BRAF and ALK. This finding suggests that ROS1 and RET are independent oncogenic drivers and could be potential druggable targets (Gainor and Shaw, 2013; Ju et al., 2012; Kohno et al., 2012; Takeuchi et al., 2012; Wang et al., 2012).

ROS1 and RET rearrangements have been identified in 1–2% of NSCLC cases (Gainor and Shaw, 2013). Patients with ROS1 rearrangement possess many of the features seen in ALK and EGFR positive patients. For example ROS1 rearrangements were associated with younger age, non-smoking history, Asian ethnicity, advanced stage and adenocarcinoma. In contrast, patients with RET rearrangements have fewer shared features with ALK or EGFR positive patients than do patients with ROS1 rearrangements (Bergethon et al., 2012; Ju et al., 2012; Kohno et al., 2012; Li et al., 2012; Takeuchi et al., 2012; Wang et al., 2012). Detection of these rearrangements can be performed by using FISH, reverse transcription polymerase chain reaction (RT-PCR), and immunohistochemistry (IHC). However, to date, no gold standard screening technique is available (Gainor and Shaw, 2013; Ju et al., 2012; Kohno et al., 2012; Li et al., 2012; Takeuchi et al., 2012).

Since ROS1 and ALK share a high degree of homology within their tyrosine kinase domains, it is possible to hypothesize that ALK tyrosine kinase inhibitors may also inhibit ROS1. Accordingly, current studies have shown that Crizotinib can be useful in patients with NSCLC, but clinical trials in patients with RET rearrangements are clearly needed (Bergethon et al., 2012; Davies et al., 2012; Gainor and Shaw, 2013; McDermott et al., 2008; Yasuda et al., 2012). Other rearrangements in RTK such as AXL and NTRK1 (neurotrophic tyrosine kinase receptor type 1) have been discovered, but the incidence of these alterations is rare and their clinical importance unclear (Shinh et al., 2005; Vaishnavi et al., 2013; Zhang et al., 2012).

### 3.7. Targeting of angiogenesis

Bevacizumab (Avastin), a humanized monoclonal antibody against Vascular endothelial growth factor-A (VEGF-A), is used to prevent angiogenesis in several human cancers include colorectal, lung, breast, and ovarian cancer (Lambrechts et al., 2013). VEGF-A

is a multi-functional cytokine which plays a major role in both inflammation and angiogenesis, the expression of which is elevated in different types of neoplasms (Carmeliet and Jain, 2011). There is no reliable marker that predicts the response to Bevacizumab (Lambrechts et al., 2013).

### 3.8. Targeting cancer stem cells

Cancer stem cells (CSCs) share many properties with normal stem cells such as self-renewal and capacity of differentiation. CSCs also have a potential to form tumors following transplantation (Clevers, 2011; Pardal et al., 2003; Valent et al., 2012). Recent in-vitro and clinical evidence supports a critical role for CSCs in tumor initiation, heterogeneity, metastasis, relapse and drug resistance (Klonisch et al., 2008; Shackleton et al., 2009; Valent et al., 2012). CSCs, therefore, represent an important novel target for cancer treatment.

CSCs associated with NSCLC express the same phenotypic markers as other CSCs namely expression of CD133, CD166 and CD44, elevation of aldehyde dehydrogenase (ALDH) activity and elevated nuclear β-catenin (Bertolini et al., 2009; Eramo et al., 2007; Huang et al., 2009; Jiang et al., 2009; Malanchi et al., 2008; O'Flaherty et al., 2012). In addition, the Wnt, Hedgehog, and Notch signaling pathways within CSCs have critical roles in controlling of self-renewal and developmental pathways in normal and cancer stem cells (Fan et al., 2006; Ingham and McMahon, 2001; Liu et al., 2006; Logan and Nusse, 2004; Miele, 2006; Reya and Clevers, 2005). However, some features of CSCs such as quiescence, expression of ATP binding cassette transporters (ABC), resistance to DNA damage, high expression of anti-apoptotic proteins and the broad expression of CSC markers in normal cells complicate molecular targeting of these cells (126). The ability to target CSCs with specific antibodies or small molecules will be dependent upon further characterization of their phenotype to establish unique genetic changes and key receptor and/or metabolic pathways (126).

## 4. Conclusion

The genetic changes that occur in cancer cells enable the dysregulation of oncogenic and tumor suppressor genes and the overexpression or activation of genes that drive cancer growth. These genetic changes frequently target a relatively few oncogenic driver pathways although differences in mutations can vary even between cells within a single cancer. The structural changes in membrane RTKs caused by these genetic changes and by oncogene addiction drive tumour cell proliferation and have provided opportunities for targeted therapy. In conjunction with the take-up of adaptive design in clinical trials, this has proved a powerful strategy towards the goal of personalized medicine. Thus pharmacogenomics by linking selected drug therapy to molecular analysis has resulted in a remarkable increase in the survival rate and quality of life for many patients with NSCLC.

However, resistance to targeted therapy exists and is often acquired after the onset of therapy and highlights the fact that cancer is a moving target. This emphasizes the need to better define and target the critical nodes within each cancer cell that controls proliferation, differentiation, apoptosis, and growth pathways. The advent of next generation sequencing linked to the development of better drugs should enable the selective treatment of patients with monotherapies or, more likely, drug combinations which will prevent the onset of resistance to monotherapies. Future studies will further increase our understanding of cancer biology and the mechanisms of resistance. It is only by being armed with this knowledge that we will be able to defeat cancer by predicting its next move.

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