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REVIEW

Personalized treatment options in Non-small Cell Lung Cancer

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Lung cancer is the leading cause of cancer related death among both men and women worldwide [1-3]. There are two major groups of lung cancer based on the histological features and response to therapy; non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC is also divided to the histological subtypes, and which accounts 80% of lung cancer patients [3, 4]. Despite advances in diagnosis, 5-year survival rates are approximately 15% for all cases [5]. Since EGFR (epidermal growth factor receptors) is overexpressed in more than 80% of NSCLC patients, its overexpression is correlated with poor prognosis and chemoresistance. However, only 10% of EGFR1 overexpressing patients respond to EGFR1 TKI (tyrosine kinase inhibitor) therapy implying that EGFR1 overexpression may not be the main factor responsible for NSCLC development [6, 7]. Therefore, new therapeutic strategies that specifically target other molecular pathways must be considered as alternative options. In this review, we tried to summarize the most recent studies in treatment of NSLC, and made suggestions on the basis of our results and clinical studies.

Keywords: lung cancer; EGFR; tyrosine kinase inhibitor; Treatment

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EGFR is a member of the receptor tyrosine kinase super family. It has significant roles in the development and growth of many cancers. The EGFR superfamily is composed of four distinct transmembrane proteins, such as EGFR (ERBB1 or HER1), ERBB2 (HER2), ERBB3 (HER3) and ERBB4 (HER4). While ERBB2 does not have ligand binding activity and ERBB3 lacks tyrosine kinase activity, other receptors carry both of these functions [8-10]

After the binding of ligands, such as, epidermal growth factor (EGF) and transforming growth factor-α (TGFα), EGFR dimerizes with the other family members either as a homodimer or heterodimer. This dimerization leads to the autophosphorylation of TK (tyrosine kinase) domain, recruitment and phosphorylation of various intracellular substrates, including the members of RAS-/RAF-/ERK-/MAPK, PI3K-AKT pathways and STATs (the signal transducer and activator of transcription proteins) proteins [11, 12]. The activation of these pathways promote several cellular responses such as proliferation, survival, differentiation, migration and adhesion [13, 14] (Figure 1)

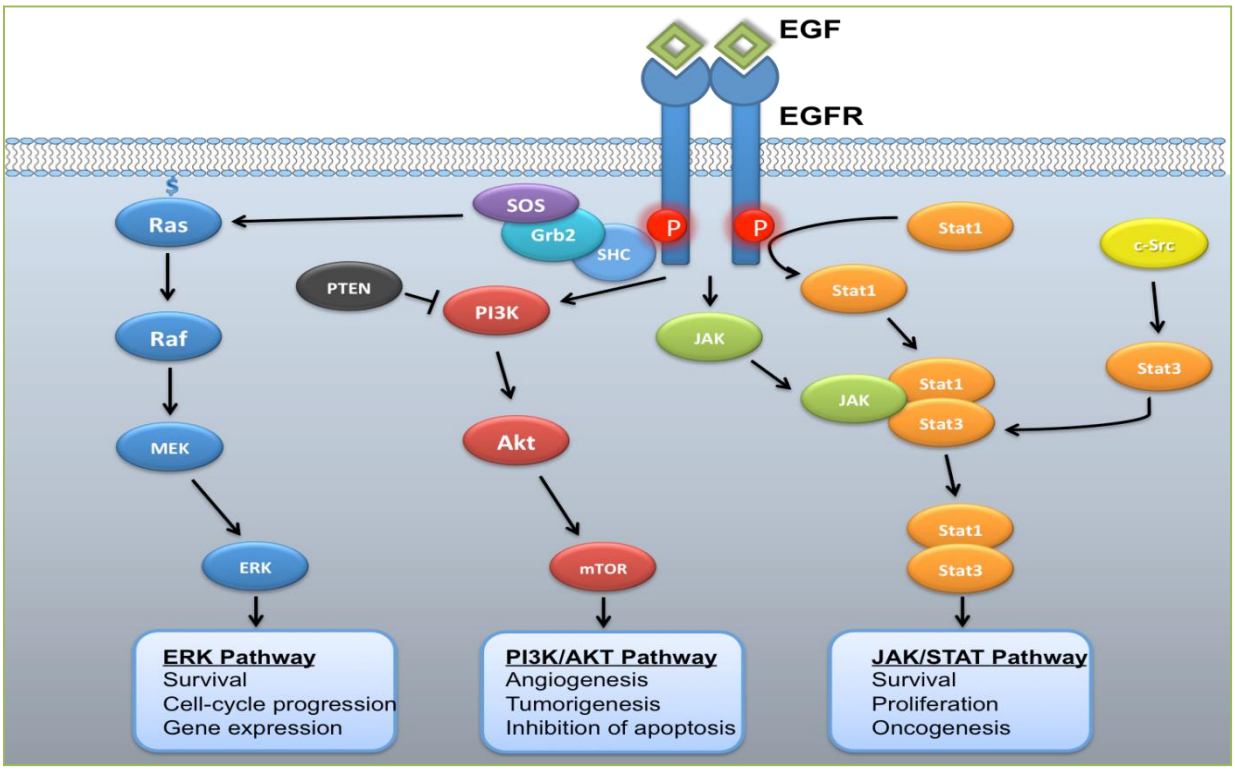

Figure 1. EGFR signaling pathway.

The EGFR pathway can be activated by various means including, EGFR overexpression, gene amplification, receptor and effector mutations, increased expression of ligands and downstream pathway proteins [15, 16]. Ten percent of SCLC have been associated with EGFR's somatic gain of function mutations [17-19], there are four types of mutations located in four exons of the EGFR tyrosine kinase domain. These are deletions in exon 19, insertions in exon 20, missense mutation (L858R) in exon 21 and the codon change of G719 [20, 21]. L858R point mutation in exon 21 and short or in frame deletions in exon 19 are the most common mutations (accounting for 82%) in NSCLC patients [22, 23] (Figure 2). These mutations are frequently seen in cases of adenocarcinomas, females, nonsmokers, and people of Asian descent [24]. However, these results do not mean that smoking have a protective effect on EGFR mutations. The above mutations are referred to as "activating mutations". They are clustered around the ATP binding cleft of the EGFR kinase domain Because of their localization, mutations stabilize the interaction with ATP leading to hyperactive EGFR signaling [3, 25].

EGFR protein activation is not only caused by mutations, but also by gene copy numbers [26]. The EGFR copy number is increased by amplification and high polisomy. Clinical studies suggested that more than 40% of NSCLC patients show more than four copies (high polisomy) of the EGFR gene [2, 20, 27]. However, it is not clear that polysomic status shows the active form of protein. EGFR gene amplification is strongly associated with EGFR mutations [9, 20, 28]. Yatabe *et al*. reported that EGFR mutations are precursors of amplifications [29]. This suggest that the EGFR may be amplified during the progression of cancer [15].

Because of these alterations EGFR has become a great target for cancer therapy. In this perspective, two approaches have been developed to inhibit EGFR activation. In the first approach, monoclonal antibodies like cetuximab were used against the receptor-ligand interactions. In the second approach, EGFR function was inhibited by targeting its tyrosine kinase domain with tyrosine kinase inhibitors(TKI) such as Erlotinib (Tarceva) and Gefitinib (Iressa) [26]. Erlotinib and Gefitinib are the first generation EGFR kinase inhibitors that disrupt EGFR activation by the binding to the ATP pocket of tyrosine kinase domain competing with ATP [30, 31] (Figure 3). Clinical studies showed that 70-80% of NSCLC patients with an EGFR mutation respond well to TKI therapy, and 10% of patients without the EGFR mutation also positively respond to therapy [2, 32].

IPASS (Iressa Pan-Asia Study) was the first study that demonstrated the importance of the EGFR tyrosine kinase inhibitor over platinum-based combination chemotherapy in patients with the EGFR mutation. In this study, of 1217

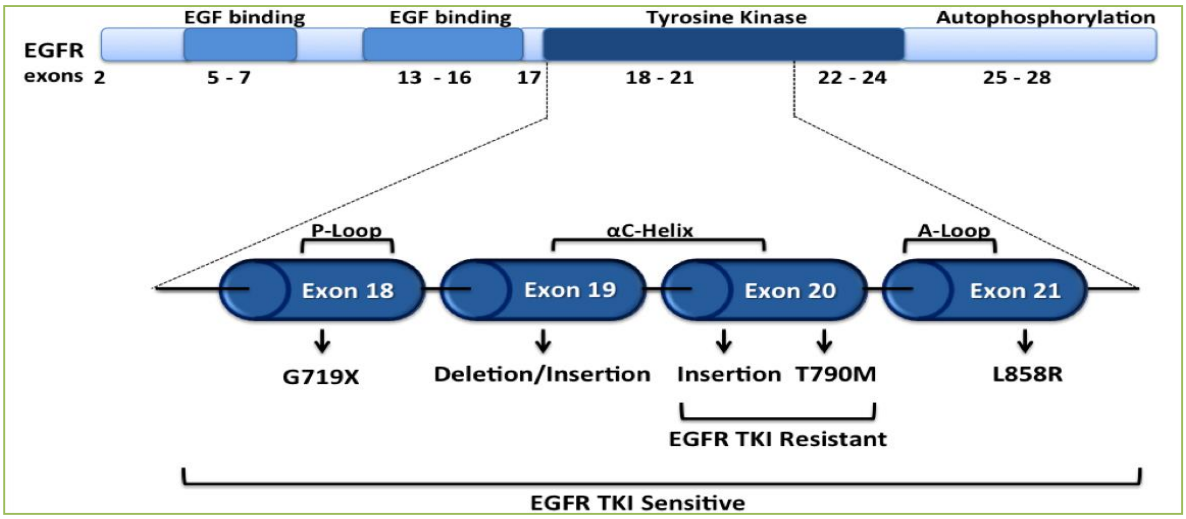

Figure 2. Common mutations in EGFR and their effects on TKI therapy.

patients, 437 had the EGFR mutation. All these patients were, chosen from East Asia, were non- smokers or, were former light smokers diagnosed with stage III-IV adenocarcinoma [33]. This study showed that response to TKI depends on the patients' gender, ethnicity, mutational status, histology and stages of tumors, smoking status and prior to chemotherapy regimens [34, 35]

IPASS and other phase III randomized studies showed that patients with exon 19 in frame deletions and/or with the exon 18 L858R mutation is sensitive to gefitinib. Whereas, exon 20 mutations are correlated with drug resistance. 261 patients who have the EGFR sensitizing mutations received Gefitinib in the IPASS study. They had an increased response rate and longer progression free survival than chemotherapy [33, 34]. These results were also confirmed in other phase III studies. Five phase III studies results are summarized in Table I. As a results of these responses, it is recommended that all patients with EGFR-mutant NSCLC receive these treatments as first line therapy [36]. All these results show that, these mutations can be used as predictive marker for the therapy response, and all patients with advanced adeno- carcinoma should be tested for EGFR mutations.[34, 36, 37]

TKIs can be used as second or third line therapy in NSCC patients who harbor wild type EGFR gene. The NCIC Clinical Trials Group BR.21 (Erlotinib) and Iressa Survival Evaluation in Lung Cancer (ISEL; Gefitinib) studies showed meaningful improve in survival and delayed worsening in the advanced NSCLC patients with wild type EGFR and nonadenocarcinoma [38, 39]. In addition to these, a variety of studies (TORCH) also suggested that TKIs can be efficient when they are used after first line chemotherapy in wild type EGFR patients [40].

EGFR gene overexpression, generally determined by immunocytochemistry-IHC, as a predictor to select patients for TKIs therapy is still debated. This selection status has been extensively investigated with controversial results. Although Capuzzo and Hirsch *et al*l claimed that there was a strong correlation between gene expression and better outcome in NSCLC patients treated with TKI, phase III studies INTEREST, INVITE and SATURN did not find any correlation between them [2, 41-43] Therefore, it is now strongly believed that detection of EGFR by IHC is not the best procedure to select patients for TKIs therapy [44, 45]

Another important parameter which can affect expression levels of EGFR is gene copy number variations. Copy number of EGFR is increased by 65% in primary NSCLC patients, and it is detected by FISH (floresance in situ hybridization) [46]. There are controversial results about its predictive value for TKI therapy. However, latest studies such as TRIBUTE, INTEREST and IPASS have not found any correlation between high copy number of EGFR and the effectiveness of TKI therapy, therefore, IPASS results clearly concluded that increased copy number shouldn't be used as a predictive marker alone for the first line TKI therapy [33, 43, 47].

After TKI's therapy, the main problem patients' faces is development of resistance. Clinical studies showed that patients who are sensitive to erlotinib/gefitinib developed resistance 12 months after therapy [48, 49]. One of the mechanisms that cause resistance is secondary mutations in EGFR. The most common mutation seen is T790M in exon 20 which is located in the ATP binding pocket of EGFR. This change was detected in approximately 50% of NSCLC patients who developed resistance to TKI therapy [21, 37]. It is very rare to detect this mutation in NSCLC

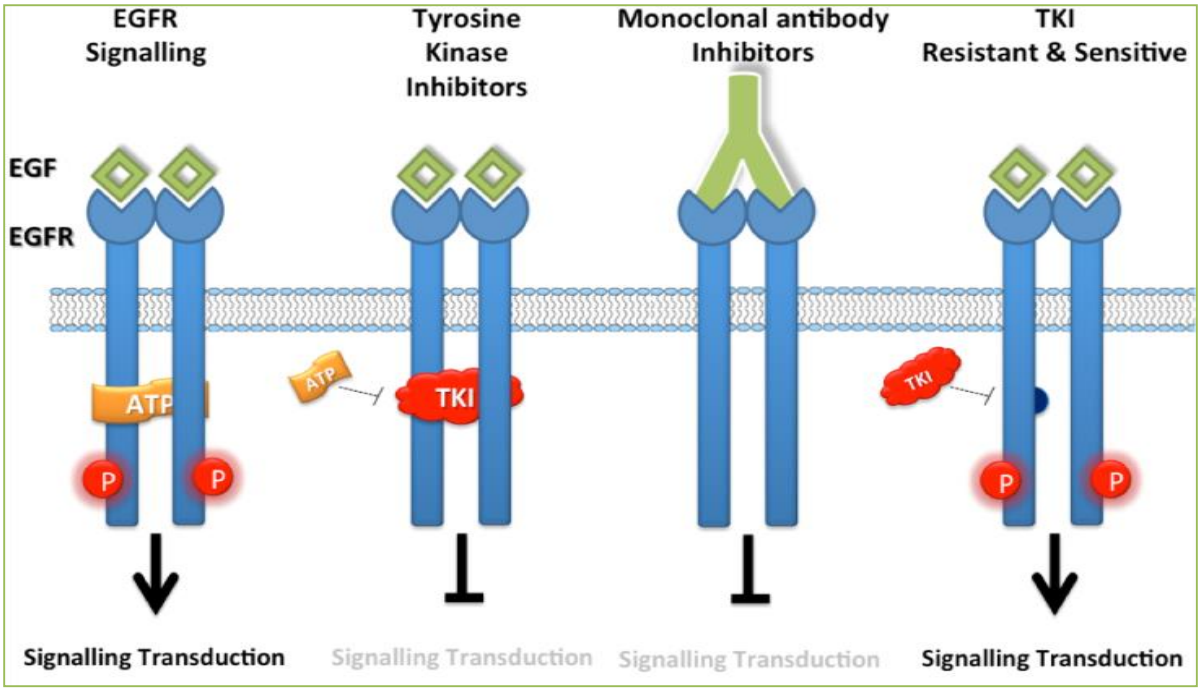

Figure 3. Mechanism of TKI and monoclonal antibody therapy.

patients who never received TKI therapy so it strictly correlated with development of resistance. Crystal structure modeling studies showed that this mutation does not affect the catalytic activity of EGFR, however, stereotypically it hinder the binding of these two drugs to ATP-binding pocket of EGFR[20, 37].

Another secondary mutation which causes resistance to TKI therapy is D761R change. This mutation is not as common as T790M, and can be inherited. It is located in alfac helix of EGFR. Balak *et al*. showed that NSCLC patients who have both L858R and D761R mutations respond to gefitinib poorly whereas NSCLC patients respond to low level of Gefitinib when these mutations occur alone [50].

Discussed below are other mechanisms which are downstream mutations of EGFR and amplifications.

Alterations in Downstream elements of EGFR in NSCLC

K-RAS mutations and clinical importance in NSCLC

The development of TKI therapies and their strong correlations with the specific predictors led researchers to define the characteristic of NSCLC and other factors that can influence the therapy responses. Defining the molecular signaling of NSCLC is also important to identify new targets and therapeutic approaches.

In this perspective, K-RAS is one of the downstream element of EGFR signaling and its activation leads to survival, proliferation and differentiation of tumor cells. The most frequent mutation observed in k-RAS is in codon 12 (G12X) which is located in GTP-binding pocket of k-RAS and generates a constitutively active k-Ras [11, 51]. These mutations in codon 12 change affinity of RAS to bind to different downstream elements. For example, G12C and G12V changes leads to activation of RAL signaling whereas G12D changes leads to activation of AKT pathways. Hence, amino acid substitution in k-RAS determines which pathway is activated [52]. These mutations occur in 30% of human cancers, and detected in 15-30% lung adenocarcinoma patients, and in current or former smokers (25%) than in never smokers (6%). Therefore, analyzing K-RAS mutational statue is very important to determine therapeutic approaches and that can be used as predictive factor to choose the right therapeutics [23].

Generally in NSCLC patients there is no association between K-RAS alterations and EGFR alterations. It is too rare to find both of the mutations in one patient [32]. This results suggests that K-RAS mutations are as important as EGFR mutations in tumorigenesis [51]. These mutations are associated with poor prognosis in NSCLC patients, and they are major predictor to chemotherapy, and associated with resistance to TKI therapies [53]. Although several studies suggested that K-RAS mutation can be predictive marker of chemotherapy, the results from these studies were not easy to analyze because of the difference in patients clinics and criteria of samples. Therefore, K-RAS

Table 1. Phase III trial studies of TKI for EGFR mutated patients (PFS: Progression free survival)

Study Name	Type	Targeted Agents	Number of patients	Response Rate	PFS	Reference
IPASS (nonsmoker or former light smoker with adenocarcinoma, East Asian, chemotherapy naïve)	Phase III	Gefitinib	132	71.2%	9.5 months	Mok *et al*[33]
		Carboplatin& Paclitaxel	129	47.3%	6.3 months	
NEJ002 (Japanese, EGFR sensitizing mutation positive, chemotherapy naïve)	Phase III	Gefitinib	114	73.7%	10.8 months	Maemondo *et al*.[80]
		Carboplatin& Paclitaxel	110	30.7%	5.4 months	
WJTOG3405 (Japanese, EGFR sensitizing mutation positive, chemotherapy naïve)	Phase III	Gefitinib	86	62.1%	9.2 months	Mitsudomi *et al*.[81]
		Docetaxel& Cisplatin	86	32.2%	6.3 months	
OPTIMAL (Chinese, EGFR sensitizing mutation positive, chemotherapy naïve)	Phase III	Erlotinib	82	83%	13.1 months	Zhou *et al*.[82]
		Carboplatin& Gemcitabine	72	36%	4.6 months	
EURTAC (Non-Asian, EGFR sensitizing mutation positive, chemotherapy naïve)	Phase III	Erlotinib	86	63%	9.7 months	Rosell *et al*.[83]
		Cisplatin & Docetaxel or Gemcitabine	87	18%	5.2 months	

mutations cannot be used as a predictive marker for adjuvant chemotherapy [30, 54].

In order to understand the role of K-RAS mutations in TKI therapy, meta-analysis and clinical studies have been done. Phase III trial BR.21 on an unselected population showed that patients with K-RAS mutations have worse prognosis, shorter survival and not respond to Erlotinib therapy [55]. Eberhard *et al*. showed that 25 patients with K-RAS mutations treated with chemotherapy plus Erlotinib had significantly shorter survival than 30 patients with K-RAS mutations treated with chemo- therapy only [56]. Similar to this, results of Meta-analysis done by Linardou and Mao suggests that K-RAS mutations are negative predictors of response to single- agent EGFR TKIs in advanced NSCLC [57, 58].

Another approach to the treatment of K-RAS mutant NSCLC patient is using MEK inhibitors [54]. These inhibitors targets MEK1 and MEK2 which are serine-/threonine kinases, and their only target is ERK1/2. Because of having one target, they are a great candidate for therapeutic markers. MEKi don't compete with ATP, they just bind to MEK proteins, and inhibit their association with ERK1/2 [59]. Selumetinib (AZD6244; Astra-Zeneca, Wilmington, DE) is one of the MEKi, and phase II trial results of this inhibitor showed that NSCLC patients treated with Selumetinib and chemotherapy had better progression free survival (PFS, 5.3 and 2.1 months) compared chemotherapy alone. The adverse events rate was in acceptable range but slightly higher in patients treated with both agents. However, they didn't get any significant data about overall survival rates [60]. In addition to Selumetinib, other MEK inhibitors are in clinical development including Trametinib (GSK1120212; GlaxoSmithKline, Philadelphia, PA), GDC-0973 (Genentech, South San Francisco, CA), and Pimasertib (AS703026 or MSC1936369B; EMD Serono, Rockland, MA).

Although initial clinical trials are producing promising results, there are some short falls against the use of MEKi on NSCLC patients who have mutant k-RAS. One of them is cytostatic effect of MEKi, so they just inhibit proliferations of cancer cells but cannot induce apoptosis. In the second mechanism, RAS mutant tumor cells exposed to MEKi switch their main proliferation pathway

from MEK-ERK to PI3K-AKT to survive. In the third mechanism, other genetic alteration such as PTEN-inactivating mutations cause constitutive activation of PI3K and LKB1 (liver kinase B1) pathways leading to resistance or reduced sensitivity to MEKi therapy. Because of all these reasons, combinational therapies seem to be more useful in RAS mutated cancers [54, 59, 61].

PI3K/AKT mutations and clinical importance in NSCLC

PI3K/AKT is one of the most important path-way for progression and metastasis of the tumor cells. Although this pathway is one of the major downstream target of EGFR signaling, it can be activated even in the absence of overexpression or activation of EGFR as shown by us recently [62]. According to our results, AKT1 is overexpressed/activated in nearly 60% of NSCLC patients with poor prognosis. This overactivation/expression could be result of aberrant activation of several mechanisms such as activating mutations in EGFR or other growth factor receptors, activating mutations of RAS members, amplification of PI3K/AKT, and loss of PTEN function. Considering the rate of PI3K activating-mutations (1-3%) and amplifications (12%- 17%) in NSCLC patients our results cannot be explained by these [23, 30]. Therefore, there must be an activation of multiple pathways converged on PI3K activation. The most common PIK3CA mutations are detected in exon 9 (E542K and E545K), which that encodes the helical domain of p110□ and exon 20 (H1047R), which encodes the catalytic domain of p110□. Both of these mutations and amplifications in PIK3CA lead to activation of AKT pathways without ligands [63].

Ludovini *et al*. showed that PI3KCA mutations are correlated with resistance to TKIs therapies and patients with these mutations had decreased overall survival and shorter median time to progression [64]. On the other hand, NSCLC cell lines which are resistant to Gefitinib showed PI3K overactivation without any mutation. This result suggests that targeting PI3K with EGFR may be useful to get better response TKI resistant NSCLC patients [65].

Recent advances in NSCLC treatment have focused on personalized therapy, and inhibitors have been developed to target the PI3K mutants. There are three class of inhibitors that target the PI3K pathway; Pan inhibitors, combined PI3K/mTOR inhibitors and AKT inhibitors [66]. Pan-inhibitors target the catalytic domain of PI3K. PX866 is one of the wortmanin derivatives and efficiency of pan-PI3K inhibitors was determined by phase II trial in combination with docetaxel in patients with NSCLC [65].

GDC-0941 (Genentech) is another PI3K inhibitor which binds to ATP binding site of PI3K, it is orally available and tested in phase I trials, combined with chemotherapeutic agents and with/without anti-VEGF antibody against solid tumors including NSCLC. Results of this study showed that its treatment leads to decrease in pAkt and pS6 level in tumor samples [67]. Therefore, GDC-0941 seem to be a promising drug candidate for targeting PI3K.

BKM12 (Novartis) inhibits all class-I PI3Ks and especially targets common mutations of PIK3CA. Preclinical datas showed that its biological activity is correlated with pAKT inhibition in cell lines and xenograft models. In combinational therapy of BKM120 with mTOR inhibitors also caused inhibition of growth of NSCLC cell lines and murine lung xenograft models [68, 69]. There are ongoing projects in Phase I/II trials on NSCLC patients.

XL147 (SAR245408) is also one of the clinical studies that target PI3K. Phase I trial in combination with paclitaxel and carboplatin in adults with solid tumors including NSCLC patients is still ongoing and another study combining with erlotinib has completed. Treatment with this combination stabilized disease through inhibition of PI3K and EGFR pathways [70, 71].

NVP-BEZ235 is another inhibitor which target both PI3K/mTOR. This drug candidate is a competitorinhibitor for ATP. According to preclinical studies combination of NVP-BEZ235 therapy with MEK inhibitors on murine lung adenocarcinoma models with PI3K or KRAS mutations cell line reduced tumor sized gle therapies with this compound did not benefit. Therefore, combinational therapies with PI3K inhibitors are important especially in patients who have K-RAS mutations. [72-74].

AKT isoform mutations have been identified human cancers, but, only AKT2 gene mutations have been detected in 2.5% of NSCLC patients [4, 75]. This result suggested that mostly post translational changes are responsible for AKT activation. AKT activation may be resulted in PI3K pathway activation or it is activated by itself [70]. Tumor samples from NSCLC patients with metastasis showed increased pAKT levels, and this is correlated with poor prognosis and resistance to chemo/radiotherapy [76]. In support of this, patients whose tumors were negative for p-AKT had a better response rate to TKI, disease control rate, and time to progression. However, these parameters did not differ according to p-ERK levels [4, 62].

AKT may be a great target because of overexpression and activation in NSCLC. There are some AKT inhibitors which target ATP binding site or pleckstrin homology (PH) domain within the protein. MKK-2206 (Merck) is one of the first inhibitor targeting AKT1/2/3. It inhibits the protein through binding its PH domain, homology domain. Preclinical studies showed that use of this drug in

combination with chemotherapeutics suppressed AKT activation and inhibited the survival of lung cancer cell lines [77, 78]. When it was used with erlotinib, it resensitized erlotinib resistance NSCLC to the therapy [79]. Even though, preclinical studies produced some promising results, use of AKT inhibitors against cancer will be more challenging than any other inhibitors, because AKT regulates lots of metabolic pathways, so its inhibition causes metabolic toxicities such as insulin resistance, drug-induced hyperglycemia and hyperinsulinemia [65] .

Conclusions

Currently, EGFR1 is still the most important diagnostic marker for NSCLC, and this is determined by IHC. However, even if IHC results are positive this does not say much about activation status of EGFR1 and its downstream elements. Since only 10% of EGFR1-positive patients respond to TKI therapy, this clearly indicate that EGFR1 overexpression may not be the main cause of NSCLC development in the remaining patients. Supporting this, we have recently shown that tumor samples derived from NSLC patients can show robost activation of AKT, ERK, and STAT3 while EGFR1 is not activated in the same samples. Therefore, we are suggesting that at least the activation (phosphorylation) status of downstream elements of PI3K and RAS pathways must be determined in tumor samples. We are suggesting this because inhibitors of these pathways are being tested in clinical trials, and can be used in selected populations.

Conflicting interests

The authors have declared that no competing interests exist.

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