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Review

EGFR-Targeted Therapy for Non-Small Cell Lung Cancer: Focus on EGFR Oncogenic Mutation

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Abstract

The two essential requirements for pathologic specimens in the era of personalized therapies for non-small cell lung carcinoma (NSCLC) are accurate subtyping as adenocarcinoma (ADC) versus squamous cell carcinoma (SqCC) and suitability for EGFR molecular testing, as well as for testing of other oncogenes such as EML4-ALK and KRAS. Actually, the value of EGFR expressed in patients with NSCLC in predicting a benefit in terms of survival from treatment with an epidermal growth factor receptor targeted therapy is still in debate, while there is a convincing evidence on the predictive role of the EGFR mutational status with regard to the response to tyrosine kinase inhibitors (TKIs).

This is a literature overview on the state-of-the-art of EGFR oncogenic mutation in NSCLC. It is designed to highlight the preclinical rationale driving the molecular footprint assessment, the progressive development of a specific pharmacological treatment and the best method to identify those NSCLC who would most likely benefit from treatment with EGFR-targeted therapy. This is supported by the belief that a rationale for the prioritization of specific regimens based on patient-tailored therapy could be closer than commonly expected.

Key words: EGFR targeted therapy, NSCLC, advanced, mutation, TKIs, resistance.

Introduction

Alterations in receptor tyrosine kinases (RTKs) including over expression, amplification or mutation have shown to play a key role in the pathogenesis of lung cancer[1]. In recent years, attention has been paid to the role that "driver mutations" have in tumorigenesis, in order to use them as potential targets for therapy. Such "driver mutations" include those of the epidermal growth factor receptor (EGFR) and of the anaplastic lymphoma kinase (ALK) [2,3].

The EGFR family of TKs, referred to as the HER or ErbB family, consists of four members – EGFR (HER1/ErbB1), HER2 (ErbB2), HER3 (ErbB3) and

HER4 (ErbB4). These members regulate many physiological processes and are involved in the modulation of cell proliferation, apoptosis, cell motility and neovascularisation, thus being able to induce important mechanisms related to cancerogenesis[4,5]. The EGFR tyrosine kinase works through the auto-activation of the receptor via its homo/heterodimerization and autophosphorylation on tyrosine-rich cytosolic domains after the binding of the ligand. This leads to the beginning of two main downstream intermediate pathways: the PIK3CA/AKT1/MTOR pathway and the RAS/RAF1/MAP2K1/MAPK1 kinases[6]. There is evidence that the activated EGFR can also mediate signals through the STAT transcription factors[7,8]. Improper activation and over-expression of EGFR-TK results in increased cell proliferation, survival, invasion and metastasis. This has been implicated in the pathogenesis and progression of many malignancies as well as in the poor prognosis of patients [7,9-10]. In malignant cells, including NSCLC cells, the activity of the receptor may become dysregulated and no longer under the control of inherent inhibitory mechanisms[11].

Spontaneous EGFR mutations often are oncogenic; that is, they activate the EGFR-signalling pathway in the absence of ligand and promote cell proliferation, survival and anti-apoptotic signals. These signalling networks make EGFR-mutated cells dependent on a functional EGFR for their survival, rendering them addicted to the receptor. Inhibition of EGFR leads to up-regulation of pro-apoptotic molecules and finally results in cell death through the activation of the intrinsic mitochondrial apoptotic pathway[12,13]. There are several described mutations in the EGFR gene. The two most common are: 1) short in-frame deletions around the LREA motif of exon 19 (~45-50% of mutations); and 2) a point mutation (CTG to CGG) in exon 21 that results in substitution of leucine by arginine at codon 858, L858R (~45-50% of mutations)[14,15]. These mutations are more frequently found in NSCLC with an adenocarcinoma histology, tumors in women, East Asians and never smokers[14-16].

EGFR mutations in lung cancers constitute one of the major subsets among those molecular aberrations occurring in lung cancers. The incidence of EGFR mutations in tumors with non-small-cell histology ranges from ~15% in Caucasians to ~50% in East Asians[17]; 95% of such mutations have been found in adenocarcinomas[18]. Patients bearing EGFR mutations have shown favourable clinical outcomes even with conventional chemotherapy suggesting that EGFR may serve as a predictive factor as well as a prognostic factor[19].

Over 50% of patients diagnosed with NSCLC present with stage IIIB or IV disease is not amenable to curative treatment[20] and the only pathologic material guiding systemic therapy may be small biopsy and cytology specimens.

Until the recent use of TKIs, the standard first-line treatment for most patients with unresectable NSCLC and good performance status has involved the use of a combination of chemotherapy regimens (usually cisplatin-based), which from the 1970s and 1980s were shown to reproducibly achieve objective response in 20% to 30% of advanced NSCLC patients. The most common combination regimens in use at present are gemcitabine with either cisplatin or carboplatin, followed by paclitaxel-carboplatin, vinorelbine-platinum and docetaxel-platinum combinations[21,22]. The addition of the recombinant humanized monoclonal antibody bevacizumab that binds to vascular endothelial growth factor (VEGF) to carboplatin and paclitaxel for the treatment of non-squamous advanced NSCLC has demonstrated to increase RR, PFS and OS when compared to chemotherapy alone[23]. Disease progression affects almost all patients after initial treatment and requires additional therapy. The agents approved for second-line therapy in advanced NSCLC are docetaxel[24], pemetrexed[25] and erlotinib[26]. When tested in randomized trials[24-26], these agents have demonstrated a PFS below 2-3 months with a median overall survival no longer than 9 months in very few unselected patients.

Despite recent advances with approval of more active chemotherapeutic and anti-angiogenesis agents for stage IV NSCLC, standard therapy can provide only modest clinical benefits with significant toxicities when used in unselected patients. In 2004, the identification of somatic mutations in the EGFR gene provided the first glimpse of a possible target for a treatment[27,28] which could maximize clinical outcome in those patients who could benefit from a personalized therapy[29]. This implies the identification of certain characteristic molecular lesions meant to be causally responsible for maintenance of the malignant phenotype and also distinctive of the cancer cells. Therapies targeted to these molecular lesions offer the prospect for tumor control and selectivity with less toxicity than traditional chemotherapy.

This review is designed to shed light on the rationale supporting the preclinical molecular footprint assessment, on the progressive development of specific pharmacological treatments and on the best method to identify those NSCLCs which would most likely benefit from treatment with EGFR-targeted therapy.

Rationale of EGFR assessment in Non Small Cell Lung Cancer

Somatic mutations in the EGFR gene which account for oncogenesis in NSCLCs are activating mutations that work through a "gain of function" mechanism (i.e. the ligand-independency of the tyrosine kinase's signalling activity). These types of mutations can be found in 10 to 20% of patients with a NSCLC at an advanced stage and in more than 50% of adenocarcinomas and tumours from East Asians, never smokers and women[30]. The described mutations generally interest the exons of the EGFR gene between the 19th and the 21th, which correspond to the portion of the receptor with kinase activity, nearby the binding site of ATP (that is fundamental to the activation of the receptor by autophosphorylation). As a result, the receptor is blocked into a state of constitutive activation, signalling to the cell to proliferate and to resist apoptosis. The two most frequent genic mutations responsible for this anomaly in cell cycle are the substitution of arginine for leucine at codon 858 (L858R), exon 21, and in-frame deletions at exon 19 (del E746_A750 is the most common). L858R accounts for 45-50% of mutations while deletions at exon 19 account for another 45-50%. Other rarer mutations (5%) associated with EGFR constitutive activation are insertions in exon 20 and substitutions at the glycine residue at codon 719 in exon 18 (as the G719S mutation). Molecular studies on the receptors harbouring a known L858R mutation demonstrated a decreased affinity of the tyrosine kinase for ATP and an increase in the affinity for the tyrosine kinase inhibitors (TKI). These are drugs studied to specifically target the pathways of oncogenesis mediated by the EGFR hyper-activation and causing little harm to non-tumoral cells[30]. There is evidence supporting a different sensitivity of NSCLCs to the TKI gefitinib depending on the presence of an exon 21 mutation or an exon 19 deletion, having deletions at exon 19 been associated with a better response[31].

Furthermore, in about 30-75% of samples from NSCLCs, an EGFR over-expression[30] due to epigenetic causes (transcriptional hyper-activation), gene amplification or oncogenic viruses can be detected[7]. EGFR over-expression and the consequent augmentation of cell survival and proliferation has also been observed in premalignant lesions, showing the importance of this membrane-associated receptor in tumor growth[7]. NSCLCs that over-express both EGFR and HER2 (another member of the HER family) demonstrated an aggressive tumor cell growth. In effect, EGFR/HER2 heterodimers display a greater activity than EGFR homodimers[7]. Some studies demonstrated that the EGFR gene amplification, and thus over-expression directly correlates with a better response to tyrosine kinase inhibitors, and NSCLCs that over-express both EGFR and HER2 are more sensitive to this class of drugs than a tumor with an increased expression of EGFR alone. In this perspective, pre-treatment assessment of EGFR and HER2 gene copy number may have a prognostic and a predictive value of response to TKIs in NSCLCs[7,32], but there are no univocal opinions with regard to this topic. However, data from the recent phase 3 FLEX study on patients with advanced NSCLC underpin pre-treatment assessment of EGFR expression by IHC, for EGFR over-expression on the surface of tumoral cells proved to be predictive of better overall survival with a combined treatment of first-line chemotherapy (comprising cisplatin and vinorelbine) plus cetuximab (an anti-EGFR monoclonal antibody) relative to chemotherapy alone. Among patients with a high EGFR expression, those who were in the combined treatment group had a median OS of 12 months versus the 9.6 months of patients allocated to the chemotherapy alone arm[33]. Which strategy is better in the management of advanced NSCLCs, if the inhibition of mutated EGFR with TKIs or a combined approach with cetuximab plus first-line chemotherapy, is yet to be determined. The combination of cetuximab and a tyrosine kinase inhibitor in the treatment of those tumors which are resistant to TKIs alone is intriguing[34,35], but to date clinical data are still lacking.

EGFR expression seen in advanced lung cancers made this an attractive target for molecular intervention in this disease. Several studies **(Table 1)** have by now demonstrated dramatic improvement in response rates, quality of life, symptoms, and median progression-free-survival (by 2-5 months) with first-line EGFR-TKI therapy compared with standard platinum-doublet chemotherapy in patients with EGFR mutation-positive NSCLC[36,37]. Gefitinib and erlotinib were the first two agents to target the tyrosine kinase domain of the EGFR. Both these agents showed encouraging activity in patients with NSCLC who had been previously treated with chemotherapy in the phase I series and then in phase II trials[36,37]. This has led to their approval of the treatment in advanced NSCLC. The first two large phase III trials comparing EGFR TKIs in patients previously treated with a different first line therapy were BR.21[38] and INTEREST[39] trials. BR21 was an international phase III randomized trial comparing erlotinib with placebo in patients who had failed at least one line of cytotoxic chemotherapy. With the survival benefit shown in this trial, erlotinib has been approved for use as second or third-line therapy in NSCLC after failure with a cytotoxic

chemotherapy regimen by American and European regulatory agencies[38]. A randomised, open-label phase III trial (INTEREST)[39] of gefitinib versus docetaxel in previously treated patients established non-inferior survival in patients treated with TKIs compared with chemotherapy, thus suggesting that gefitinib is a valid treatment for pre-treated patients with non-small-cell lung cancer[39].

In contrast to the significant clinical and radiological responses seen in patients harbouring EGFR activating mutations, gefitinib and erlotinib have shown only limited activity in non-EGFR genotyped, or unselected, NSCLCs when given as first, second or subsequent lines of therapy.[37,40]. This has been reported by several prospective trials of gefitinib and erlotinib in EGFR-mutated NSCLC, which showed RRs exceeding 70% in tumors with exon 19 deletions or the L858R mutation, with PFS intervals of 6-14 months and OS times beyond 20-24 months[40-43].

During the last three years, the predictive value of EGFR mutations for use of gefitinib has been strengthened by the results of three randomized phase III trials that specifically compared TKIs used as first-line therapy with traditional platinum-based chemotherapy in patients with advanced NSCLC. In 2009 the results of IRESSA Pan-Asia Study[36,44] were presented. This trial included 1217 patients of Asian ethnicity who were never smokers or former light smokers yet had histologic diagnosis of adenocarcinoma. The trial demonstrated an improvement in PFS and RR (with no statistical difference in OS) with the use of gefitinib in EGFR-mutated tumors and, in contrast, better RR and PFS with standard chemotherapy in patients without mutations. The first phase III trial of gefitinib versus chemotherapy as initial treatment of recurrent or advanced NSCLC, based on selection of patients with known activating EGFR mutations was the WJTOG3405 trial, reported in 2010[45]. This trial documented important achievements in terms of RR and PFS with the use of TKIs. During the same year, such results were confirmed by another similar Japanese phase III trial, NEJ002[37], with RR and PFS definitely favouring the use of gefitinib in the first-line setting of metastatic EGFR-mutated NSCLC.

Table 1. Summary of first-line efficacy data of TKIs in patients with EGFR-mutated advanced NSCLC (results from randomized phase III trials of gefitinib/erlotinib versus standard chemotherapy).

Trial	patient selection	treatment/number of patients	ORR (%)	PFS (months)	OS (months)
IPASS	Asia	Carboplatin-Paclitaxel	47	6.3	21.9
(Mok *et al*. 2009)	never- or light ex-smoker	(*n*=608 total; *n*=129 *EGFR* M+) Gefitinib	71	9.5	21.6
	adenocarcinoma	(*n*=609 total; *n*=132 *EGFR* M+)	*p* < 0.001	HR 0.48; *p*<0.001	HR 1.00; *p* 0.99
WJTOG 3405	Asia	Cisplatin-Docetaxel	32	6.3	not reached
(Mitsudomi *et al*. 2010)	*EGFR* mutation	(*n*=86 *EGFR* M+) Gefitinib	62	9.2	30.9
		(*n*=86 *EGFR* M+)	*p*< 0.001	HR 0.49; *p*<0.0001	HR 1.64; *p* 0.211
NEJ 002	Asia	Carboplatin-Paclitaxel	31	5.4	23.6
(Maemondo *et al*. 2010)	*EGFR* mutation	(*n*=100 *EGFR* M+) Gefitinib	74	10.8	30.5
		(*n*=98 *EGFR* M+)	*p*< 0.001	HR 0.30; *p*<0.001	HR NR; *p* 0.31
OPTIMAL	Asia	Carboplatin-Gemcitabine	36	4.6	NA
(Zhou *et al*. 2011)	*EGFR* mutation	(*n*=72 *EGFR* M+) Erlotinib	83	13.1	NA
		(*n*=83 *EGFR* M+)	*p*< 0.0001	HR 0.16; *p*<0.0001	NA
EURTAC	Europe	Platinum-Gemcitabine/Docetaxel	15	5.2	19.5
(Rossell *et al*. 2012)	*EGFR* mutation	(*n*=87 *EGFR* M+) Erlotinib	58	9.7	19.3
		(*n*=86 *EGFR* M+)	OR 7.5; *p*<0.0001	HR 0.37; *p*<0.0001	HR 1.047; *p* 0.87

EGFR: epidermal growth factor receptor; ORR: objective response rate; PFS: progression-free survival; OS: overall survival; HR: hazard ratio; OR: odds ratio; NA: not available, NR: not reported.

Numerous small studies (mainly conducted in East-Asia) on EGFR-TKI monotherapy with gefitinib rapidly confirmed high objective response rate with this agent used in first-line setting in patients with cancers harbouring a mutation[42,43,46-49]. Based on the results of the IPASS study, gefitinib was approved for use in Europe for the initial treatment of patients with NSCLC exhibiting EGFR mutations. Confirmatory randomized phase III trials of erlotinib versus standard chemotherapy have recently been concluded in Asia (OPTIMAL trial, NCT00874419[50]) and Europe (EURTAC trial, NCT00446225[51]). The positive results

of these studies suggested that responsiveness in mutation-positive patients was not a function of ethnicity. Furthermore, Caucasian patients demonstrated a spectrum of EGFR mutational subtypes similar to those seen in East Asian patients. Gefitinib and erlotinib have shown a similar spectrum of activity, with little differences in pharmacokinetics determining a major bioavailability for erlotinib[52]. This is the only TKI which has been approved by FDA for the management of treatment-naive patients with advanced NSCLC showing EGFR activating mutations[53].

EGFR-TKIs as a class are generally well tolerated. The two most common toxicities include dermatologic and GI effects; both of which are mild to moderate, easily managed and reversible[36,37,54].

In order to determine whether an EGFR TKI or chemotherapy is the appropriate first-line therapy, the latest guidelines[55] recommend mutation testing for all patients with advanced NSCLC tumor.

All EGFR-mutated patients treated with gefitinib or erlotinib invariably develop acquired resistance to this kind of therapy[56,57] **(Figure 1)**. The most common and first identified mutation is the threonine-790 to methionine (T790M) point mutation in exon 20 which represents approximately 50% of all acquired resistance in NSCLC[58]. The development of such genetic alteration restores the EGFR TK affinity to ATP, rendering first-generation TKIs inactive[59,60]. Other secondary resistance mutations within the same gene have been reported infrequently (L747S, D761Y, T854A)[12,61-62]. All these mutations, together with T790M, have also been identified in pre-treatment tumors and, similarly, are responsible for both a lesser sensitivity and duration of response to the first generation TKIs[61,63-65]. Other mechanisms of acquired resistance include MET gene amplification (also accounting for up to 20% of pre-treatment tumoral resistances)[66], increased signalling through parallel pathways such as the ones of VEGF[67] and IGF1R[68], mutations and activation of PIK3CA[69,70] and transformation into a small-cell lung cancer phenotype[71].

Concerns have been raised about resistance to TKIs due to mutations in the KRAS gene (which encodes for a protein downstream to the EGFR in the pathway initiated by the activation of the tyrosine kinase) or due to mutations that occur in other proteins of the same cascade (e.g. BRAF[72]). Even if some studies have underlined that KRAS mutations are negative predictors of sensitivity to TKIs in NSCLCs[73], more recent research demonstrates that they are not necessarily associated with a poor response to this receptor-targeted therapy; this is contrary to the absence of EGFR mutations, which accounts for the lack of effectiveness of treatment with tyrosine kinase inhibitors[74,75].

Management of EGFR tumor resistance has become the next challenge in order to lengthen these patients' overall survival; identification of the molecular resistance mechanisms will allow for the treatment of TKI-resistant tumors. A new class of drugs, the so-called second-generation TKIs, may be able to overcome the T790M mutation resistant cell. Compared to first-generation TKIs, these molecules show higher affinity for the ATP-binding domain, form an irreversible covalent bond to the ATP-binding site and are able to stimulate other receptors (e.g. HER2). Neratinib (HKI-272), one of the three agents investigated, hasn't shown good RR when tested on patients with known T790M mutation[76], therefore further development of this drug in lung cancer has been halted. Afatinib (BIBW2992) is being investigated as part of the LUX-Lung program which aims to evaluate the use of TKI in second- or third-line treatment in patients who have acquired resistance to gefitinib or erlotinib (LUX-Lung 1, 4 and 5) as well as the use of TKIs as a first-line treatment in patients with EGFR-activating mutations (LUX-Lung 2, 3 and 6). Therapy with afatinib has demonstrated to improve the disease control rate and to prolong PFS in both LUX-Lung 1 and 2[77,78]. The G719S mutation renders the tumor less sensitive to gefitinib, while erlotinib and the second-generation TKI afatinib have proven to be effective in tumors characterized by this substitution[30]. Dacomitinib (PF-00299804) is another irreversible TKI able to target the activity of all HER TKs and has shown activity in NSCLC cell lines harbouring the T790M mutation7[9]. This molecule has been evaluated in two phase II trials: the first one was after failure of one or two chemotherapy regimens and failure on erlotinib[80]; the second one compared it with erlotinib in the second- and third-line in patients with advanced NSCLC[81]. The results of these studies seem promising and further studies will evaluate dacomitinib in upfront therapy over first-generation TKIs and in resistant EGFR-mutated tumors[82].

Pre-treatment EGFR - genotypic assessment

As the EGFR mutational profile of NSCLCs is a strong predictor of response to therapy with the highly effective TKIs[36,37], the most recent algorithms for the management of advanced NSCLCs underline the importance of EGFR molecular testing prior to the initiation of therapy[30,83], adopting either a traditional Sanger's sequencing-based analysis[30,84] or PCR assays[30]. Recent advances in genome analysis technologies lead to the use of high-throughput next generation sequencing platforms, which may become more

available in routine clinical practice in a near future[85,86]. In particular, EGFR mutations should be sought in those NSCLCs in which they occur most frequently: adenocarcinomas, NSCLCs in people who have never smoked, Asians and women[30]. A first-line treatment with TKIs should be initiated in those patients who have a positive result for EGFR mutations, otherwise a standard chemotherapy should be considered[30,83]. EGFR mutation-positive NSCLCs which also test positive for mutations associated with primary or, most frequently, secondary resistance to first-generation TKIs (first of all the T790M substitution) may respond better to newer agents like afatinib, and dacomitinib, which have demonstrated a greater effectiveness in these clinical situations, but more clinical data are required [30,56,87-89].

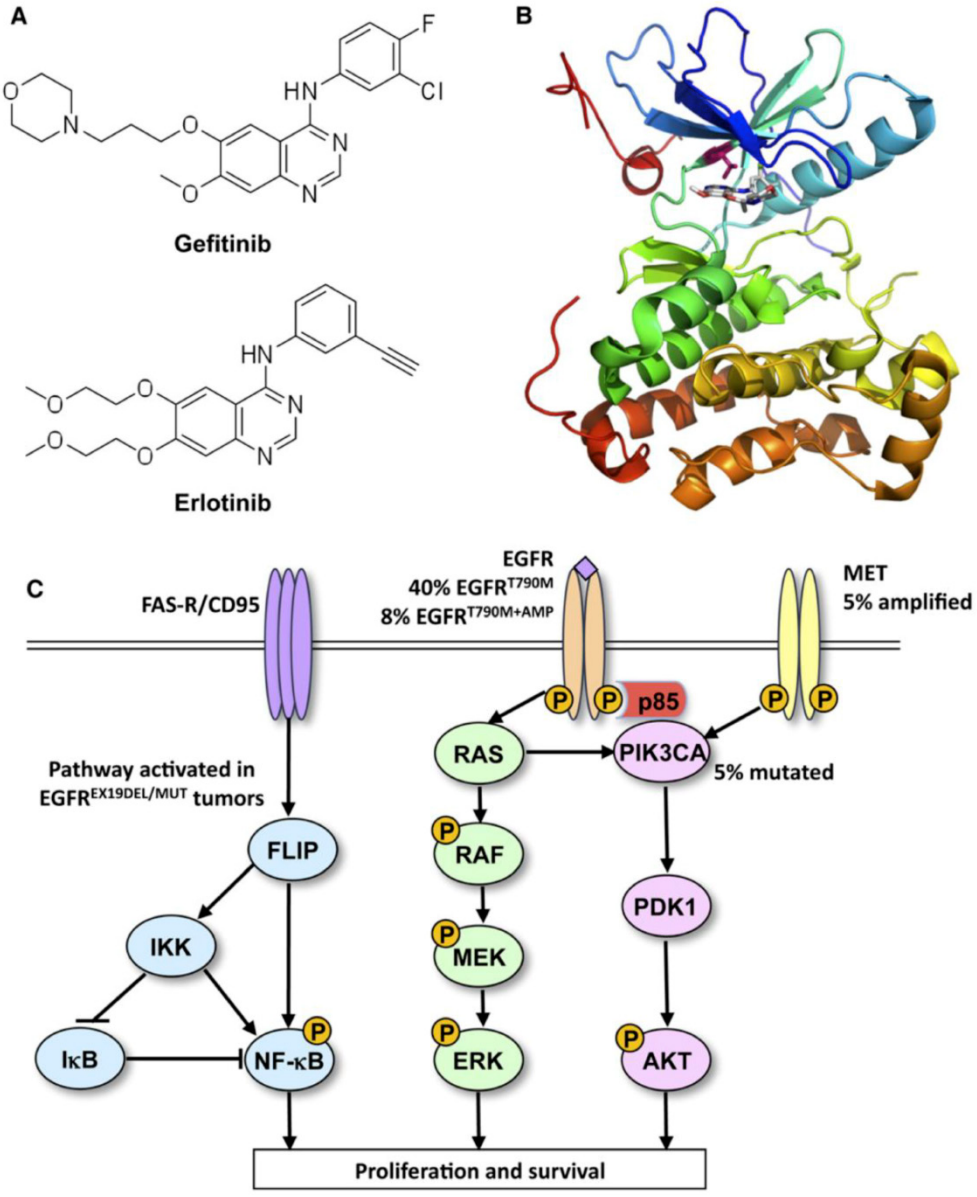

Figure 1. Ways to leave your EGFR inhibitor: Biochemical pathways leading to resistance to small molecule EGFR drugs such as gefitinib and erlotinib. (A) Structures of two approved EGFR TKIs, gefitinib and erlotinib, used in the treatment of NSCLC. (B) Ribbon diagram of wild-type human EGFR (PDB code 2ITY), illustrating binding of gefitinib to the active site of the kinase. The magenta ball-stick (located just above the gefitinib molecule in the active site) indicates the gatekeeper residue (threonine790) that is commonly mutated to methionine (T790M), resulting in reduced inhibitor binding and drug resistance. (C) Simplified pathway diagram of EGFR signaling through RAS/MEK/ERK and PI3K/PDK1/AKT indicating the points of mutation/amplification in EGFR TKI resistance as reported by Sequist and colleagues. The resistance mechanisms include the EGFR T790M gatekeeper mutation, amplification of EGFR T790M, MET amplification, and PI3KCA mutation (note that additional epithelial to mesenchymal transition changes and transformation from the NSCLC to the SCLC phenotype also lead to resistance but are not covered by this illustration). The illustration also shows the FAS/NF-kB signaling arm downstream of the FAS death receptor that was shown to be important in TKI resistance by Bivona and colleagues. Reprinted from Cancer Cell, 19, Paul Workman and Paul A. Clarke, "Resisting Targeted Therapy: Fifty Ways to Leave Your EGFR", 437-440, 2011, with permission from Elsevier.

Furthermore, some studies have supported the validity of pre-treatment measurement of EGFR gene copy number by FISH in predicting response to TKIs[7,90-92]. NSCLCs which over-express both EGFR and HER2 (over-expression directly correlates with FISH measurement of gene copy number) seem to be more sensitive to TKIs, providing another criterion that can help the clinician in therapeutic decision making[7,32]. In any case, these findings on the role of tyrosine kinase receptors' over-expression as a positive predictor of response to molecular-targeted drugs are controversial because of the conflicting evidence of different trials[55,90-92]. It is worth noting that the conflicting evidence may also be due to methodological differences among laboratories performing the FISH assay or to different interpretations of FISH results[90].

Accordingly, the only strong evidence on the effectiveness of predictors of success in the treatment of advanced NSCLCs with TKIs is in favor of EGFR mutation assessment [55,90-92].

In future, when other classes of now developmental targeted drugs are available in clinical practice, the assessment of mutations or overexpression of genes other than EGFR in EGFR negative NSCLCs (KRAS, HER2, ALK/EML4-ALK, PIK3CA, BRAF, FGFR1) may become routine[30,83].

Although genotypic assessment may be useful in determining which patients are likely to respond to EGFR TKI therapy, problems are associated with obtaining suitable DNA for pre-treatment analysis.

The quality of the samples and the extracted DNA and the quantity of DNA available using current methods may limit the routine use of genotypic assessment. As described above, cancer cells from various sources have been used for genotyping. These include archived surgically resected tissue from patients who subsequently develop recurrent or progressive disease, formaline-fixed paraffine-embedded biopsy tissue, frozen sections, cytology specimens from lavage, pleural effusion, FNA (CTCs), tumor DNA in serum and tumor DNA in plasma or blood. The mutation status of EGFR can be also determined in tumor specimens obtained during curative surgery or at biopsy. Surgery offers the best chance of high-quality and high-volume tumor tissue samples, however only 20-25% of lung cancers are suitable for curative surgery and EGFR TKI therapy is only licensed for use in patients with advanced disease.

Generally, there is only a small amount of tumor in routine diagnostic biopsy samples from patients with advanced disease; that is why specimens should be optimized and handled properly so that the most accurate pathologic characterization is achieved by individualizing among NSCLC, especially in adenocarcinoma, those histotypes that are more frequently driven by EGFR mutation. Especially for poorly differentiated neoplasms, a significant amount of specimen is often needed for diagnostic purposes and "consumed" for special immunohistochemical stains, as P63, P40 (markers of squamous differentiation), TTF-1 (positive in most of primitive lung adenocarcinoma), cromogranine and synaptophysine (marker of neuroendocrine differentiation), therefore reducing the material available for molecular testing. Lim and colleagues were able to obtain sufficient genomic DNA for genotypic assessment from more than 80% of their 24 low-volume samples (needle or forceps biopsy or fine-needle aspiration)[93]. Of the 139 patients studied by Shih and colleagues[94] only two had insufficient DNA for analysis, whereas Savic and colleagues[95] successfully sequenced the DNA from 93% of their 84 cytological NSCLC specimens. Nakajima and colleagues[96] determined EGFR mutation status in all 43 patients in their study, and Yoshida and colleagues[97] determined EGFR mutation status in all 35 fine-needle aspiration/biopsy samples in their study.

What is of crucial importance is that any tested sample is checked for adequacy in terms of the number and percentage of tumor cells. Two recently published studies reporting successful EGFR mutation detection from FNA cytology samples found EGFR mutation rates significantly lower than those reported from the same laboratories using tissue biopsies[98,99], while a recent review reported a sensitivity between 92% and 100% for EGFR mutation detection in tissue samples, varying with different techniques, and comparable results with cytology samples[100].

Follow-up biopsy to determine the T790M mutation status may be unnecessary if further research supports the initial finding that DNA sufficient for genotypic assessment can be isolated from blood samples[101-103]. A study reported a 92% sensitivity for detection of EGFR mutations in patients with metastatic NSCLC with this method (11 of 12 patients)[83].

There seems to be homogeneity of EGFR mutational state within the same tumoral lesion, even if histologically heterogeneous, and this provides a rationale for a sample-driven therapy[104]. However, it remains to be further elucidated if there are practical consequences in relation to the finding that NSCLCs' metastases in extrapulmonary organs or multiple intrapulmonary nodules can either differ from the primary lesion or from each other in regards to the EGFR mutational state[105-107] and expression[107] as detected by the laboratory. It is in this context if multiple biopsies are necessary to correctly evaluate the EGFR profile. Moreover, the response of some EGFR mutation positive patients to TKIs treatment has proven to be

"mixed"[105]: this means that clinical responses which are not completely consistent with those expected from the EGFR mutation analysis, could be explained by the aforementioned differences in the EGFR mutational state and expression among different tumoral sites.

Furthermore, it seems that chemotherapy is able to modify the EGFR expression in NSCLCs[107] by increasing or decresing it. This should be taken into consideration when anti-EGFR monoclonal antibody cetuximab is used to treat patients whose tumoral EGFR expression was assessed before chemotherapy: in this case, EGFR expression should be reassessed after having performed chemotherapy.

Conclusions

It is reasonable to suggest that personalized therapy for NSCLC patients should include a genetic assessment of the EGFR mutational status for individual patients. In this scenario, our overview aims to focus on the necessity of standardizing and improving pre-diagnostic and diagnostic tools and of optimizing the accuracy and sensitivity of EGFR mutational testing so that it might be introduced into routine clinical practice. The appropriate role of an EGFR mutation routine analysis in the treatment of patients with NSCLC continues to evolve; this on the basis of new prospective clinical studies providing new standards of care such as adequate documentation of the EGFR mutational status in the preclinical setting, during the treatment and in related follow-up.

There is reasonable basis to believe in the use of instrumental molecular reassessment of neoplastic biological characteristics for patients with advanced NSCLC throughout the clinical course in order to finely tune the treatment, particularly in the case of disease progression. In this way, TK inhibitors could be used for the best outcome in the patient, and the drug resistance that supervenes during treatment can thus be promptly identified. In addition, close cooperation between clinicians, surgeons, molecular biologists and pathologists is crucial for a continuous improvement in the field of NSCLC target therapy.

Abbreviations

NSCLC: non small cell lung cancer; ADC: adenocarcinoma; SqCC: squamous cell carcinoma; RTKs: receptor tyrosine kinases; TKIs: tyrosine kinase inhibitors; ALK: anaplastic lymphoma kinase; HER: human epidermal growth factor receptor; ErbB: erythroblastic leukemia viral oncogene homolog; PIK3CA: phosphatidylinositol 3′-kinase; AKT: protein kinase B (PKB); mTOR: mammalian target of rapamycin; RAS: rat sarcoma; RAF: rapidly accelerated fibrosarcoma; MAP: mitogen-activated protein; STAT: signal transducers and activators of transcription; VEGF: vascular endothelial growth factor; RR: response rate; ATP: adenosine-5'-triphosphate; IPASS: Iressa pan-Asia study; EURTAC: European Tarceva Versus Chemotherapy; FDA: food and drug administration; GI: gastrointestinal; MET: MNNG HOS Transforming gene; IGF1R: insulin-like growth factor 1 receptor; KRAS: v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; BRAF: v-raf murine sarcoma viral oncogene homolog B1; PCR: polymerase chain reaction; IHC: immunohistochemistry; FISH: fluorescence in situ hybridization; FGFR1: fibroblast growth factor receptor 1; DNA: deoxyribonucleic acid; FNA: fine-needle aspiration; CTCs: circulating tumor cells; SCLC: small cell lung cancer.

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Competing Interests

The authors have declared that no competing interest exists.

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