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REVIEW

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Epidermal growth factor receptor tyrosine kinase inhibitors for the treatment of non-small-cell lung cancer: results and open issues

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Abstract The medical treatment of non-small-cell lung cancer (NSCLC) has progressively changed since the introduction of "targeted therapy". The development of one of these molecular drug categories, e.g., the epidermal growth factor receptor (EGFR) tyrosine-kinase (TK) selective inhibitors, such as the orally active gefitinib and erlotinib, offers an interesting new opportunity. The clinical response rates obtained with their employment in unselected patient populations only account for approximately 10%. Because of this, over the last two years numerous studies have been performed in order to identify the patient subsets that could better benefit from these agents. Not only patient characteristics and clinical-pathological features, such as never-smoking status, female gender, East Asian origin, adenocarcinoma histology, bronchioloalveolar subtype, but also molecular findings, such as somatic mutations in the EGFR gene, emerge as potentially useful prognostic and predictive factors in advanced NSCLC. Further, specifically designed clinical trials are still needed to completely clarify these and other open issues that are reviewed in this paper, in order to clarify all the interesting findings available in the clinical practice.

Keywords Targeted therapies • NSCLC • EGFR • TK inhibitors • Prognostic and predictive factors

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Background

Lung cancer mortality accounts for almost one third of all cancer-related deaths and non-small-cell lung cancer (NSCLC) represents the most frequent histotype [1]. The role of cytotoxic chemotherapy (CTx) is limited, and the prognosis remains unsatisfactory with 5-year survival rates of only 15% for all stages. The median survival of locally advanced and metastatic NSCLC does not exceed 18 months and 9 months respectively [2]. This is why the requirement for more effective treatments with fewer side effects has provoked the most recent interest in biological and clinical research.

The epidermal growth factor receptor (EGFR) is a tyrosine kinase (TK) receptor of the ErbB family, which includes ErbB1 (HER-1 or EGFR), ErbB2 (HER-2/neu), ErbB3 (HER-3) and ErbB4 (HER-4). EGFR, a 170-kDa membrane-bound protein, encoded by 28 exons, located on chromosome 7p12, as well as the other family members, have an integral kinase activity and an extracellular ligand-binding domain, a transmembrane region and a multifunctional cytoplasmatic tail. The latter has an ATP-binding site that allows the receptor autophosphorylation, which enhances an enzymatic pathway, involved in the cellular proliferation, angiogenesis and survival. The autophosphorylation is mediated by ligand binding, which causes receptor dimerisation, both as homodimer and as heterodimer with other members of EGFR family, preferentially with HER2. This process activates downstream signalling pathways, including the Ras/Raf/Mitogen-activated protein kinase (MAPK) pathway and the phosphatidylinositol 3-kinase (PI3K)-Akt pathway, linked to cell proliferation, transformation and survival (Fig. 1).

EGFR is over-expressed in 40%–80% of NSCLC patients, suggesting that the activation of the EGFR signal pathway represents one of the essential mechanisms of tumorigenesis. This is correlated with disease progression, lower response to standard therapy, development of resistance to cytotoxic drugs and overall poor survival [3, 4].

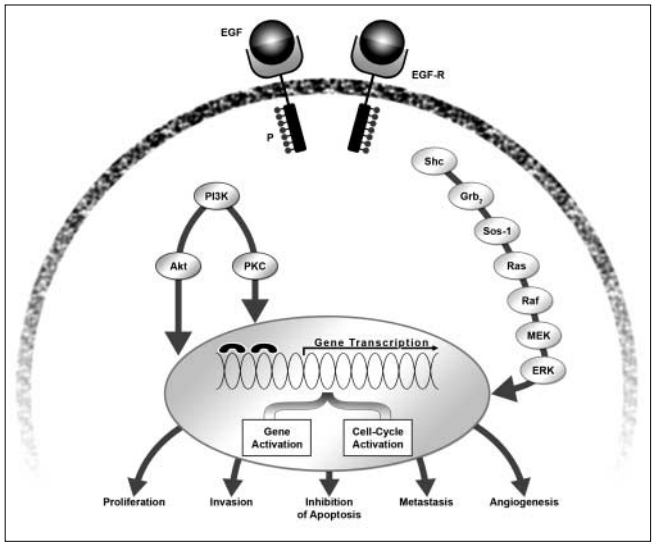

Fig. 1 EGF receptor-signal transduction pathway

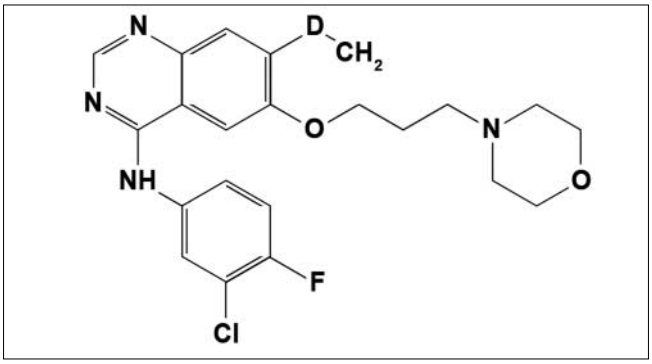

Fig. 2 Gefitinib

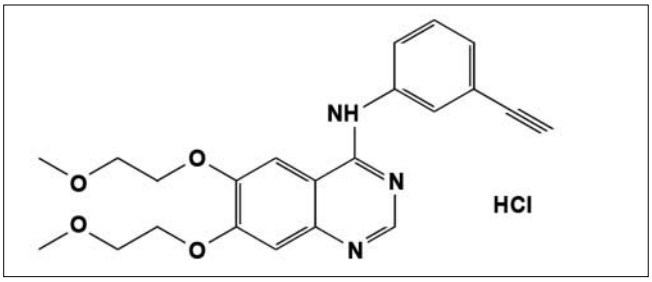

Fig. 3 Erlotinib

The identification of small molecules able to inhibit the TK activity of EGFR, by competing with ATP for the ATP-binding site, stopping tumour cell growth by preventing the activation of EGFR, represents a new form of anticancer treatment, aimed at the blockage of a special target. Currently two of these agents have been approved by the US Food and Drugs Administration (FDA): the orally active gefitinib (Fig. 2) and erlotinib (Fig. 3).

After a long series of preclinical studies, these molecules were first clinically tested in CT-refractory patients (Table 1). Two phase II studies [5, 6] were conducted in order to test the efficacy of gefitinib at two different doses: the IDEAL-1 shows 18% partial response (PR), median survival of 8 months in Japanese and European patients who received one or two prior platinum-based CTx. The IDEAL-2 shows 11% PR, median survival of 7 months in American patients who had previously received two or more regimens containing cisplatin or carboplatin and docetaxel. Disease control (objective response and stable disease) was achieved in approximately 50% of the patients in both trials, together with symptom improvement.

Two subsequent randomised phase III trials [7, 8] were conducted in previously untreated patients; a standard platinum-based CTx was employed, with or without gefitinib at two doses (INTACT-1, cisplatin and gemcitabine±gefitinib; INTACT-2, carboplatin and paclitaxel±gefitinib). The results of both studies do not show any differences in response rates, time to progression and survival with the addition of the targeted therapy to CTx.

Similar studies were conducted in order to evaluate the efficacy of erlotinib at the dose of 150 mg: this molecule was administered in previously treated patients in a phase II trial [9], producing an objective response rate of 12% and a median survival of 8 months. Two subsequent phase III trials [10, 11] randomised patients in advanced stage to receive as first-line treatment a platinum-based CTx, with or without erlotinib (TALENT, cisplatin and gemcitabine±erlotinib; TRIBUTE, carboplatin and paclitaxel±erlotinib). Both studies fail to demonstrate significant clinical benefits in the experimental arm.

Two recent phase III studies focused on the efficacy of EGFR TK inhibitors in comparison with placebo. In the Canadian trial (BR. 21) patients were randomised to receive erlotinib or placebo after first or second-line CTx [12]. Statistically significant differences were observed for overall survival and progression-free survival. A similar study (ISEL) randomly assigned patients to receive gefitinib or placebo, but it failed to show any advantage in sur-

Table 1 Drug-related non-haematological toxicities by worst common toxicity criteria (CTC) grade

Adverse event	CTC grade 1–2	CTC grade 3–4
Gefitinib, 250 mg/day, *n*=102		
Diarrhoea	47%	1%
Rash	43%	–
Acne	25%	–
Dry skin	13%	–
Nausea	12%	1%
Vomiting	11%	1%
Erlotinib, 150 mg/day, *n*=485		
Rash	67%	8%
Diarrhoea	48%	6%
Anorexia	43%	9%
Fatigue	34%	18%
Dyspnoea	13%	28%
Nausea	30%	3%
Vomiting	21%	2%

vival [13]. This data led to consideration of a possible difference between these two molecules, although the results of phase III studies differently designed could also be taken into consideration.

Safety evaluation has been primarily based on the results of trials in which patients received gefitinib or erlotinib monotherapy. From all these trials, the toxicity profile for both drugs is favourable.

No significant myelosuppression is observed. The most common non-haematological toxicities by worst common toxicity criteria grade reported at the recommended doses of gefitinib and erlotinib are shown in Table 2. They are diarrhoea, rash, dry skin, nausea and vomiting. Adverse events reported to a lesser extent were pruritus, anorexia, asthenia, weight loss, peripheral oedema, amblyopia, dyspnoea, conjunctivitis, vesiculobullous rash and mouth ulceration. Liver function test abnormalities have occasionally been observed. Cases of interstitial lung disease have been reported in patients receiving gefitinib and erlotinib with an overall incidence from all studies of approximately 1% and 0.6%, respectively [14].

In this paper we have reviewed the current knowledge and pointed out the future directions of the clinical utilisation of gefitinib and erlotinib in NSCLC.

Prognostic and predictive factors

Clinical and pathological features

The overall unexpected low percentage of clinical response has been investigated in order to identify clinico-biological characteristics potentially related to a selective activity of these agents in patient subgroups.

Analysis of data from the principal trials show that response to TK inhibitors does not correlate with EGFR overexpression, but instead, well defined patient and tumour characteristics, such as female gender, non-smoking status, East Asian ethnicity, adenocarcinoma histology and bronchioloalveolar subtype, represented the features most likely associated with treatment response.

In the IDEAL 1 trial, the response rate is higher in Japanese patients (27.5% *vs*. 10.4%, p=0.0023), is 2.5 times higher in women than in men and 3.5 times higher in adenocarcinoma than in other histologies [5]. In the IDEAL 2 trial, the response rate is 19% in the female population and 3% in the male, and 13% for adenocarcinoma *vs*. other histologies [6]. The Expanded Assess Program conducted at the Memorial Sloan-Kettering Cancer Center confirms better responses in women (19% *vs*. 8%, p=0.14) and in adenocarcinoma (19% *vs*. 0%, p=0.004), particularly in bronchioloalveolar carcinoma (38% *vs*. 14%, p=0.001), and finds a significantly higher response rate in never-smoker patients *vs*. former or current smokers (36% *vs*. 8%, p=0.001) [15].

Several smaller trials confirm the above-mentioned results, and contribute to better define the characteristics of the patient populations more responsive to this form of anticancer treatment [16].

EGFR mutations

Molecular mechanisms underlying TK inhibitor sensitivity have been recently identified, with the majority of high-

Table 2 Clinical response rate and median survival obtained with gefitinib and erlotinib in NSCLC treatment, investigated in phase II and III clinical trials

Trial, year	Phase	Treatment	Number of pts	Response rate (%)	Median survival (months)
IDEAL 1, 2003 [5]	II	Gefitinib (250 or 500 mg*)	210	18	8
IDEAL 2, 2003 [6]	II	Gefitinib (250 or 500 mg*)	216	11	7
INTACT 1, 2004 [7]	III	G/C+gefitinib (250 or 500 mg*)	730	50	10
		vs. G/C+placebo	363	45	11
INTACT 2, 2004 [8]	III	C/P+gefitinib (250 or 500 mg*)	692	30	9
		vs. C/P+placebo	345	29	10
TALENT , 2004 [10]	III	G/C+erlotinib (150 mg)	586	31	10
		vs. G/C+placebo	586	30	10
TRIBUTE, 2005 [11]	III	C/P+erlotinib (150 mg)	539	21	11
		vs. C/P+placebo	540	19	10
BR.21, 2005 [12]	III	Erlotinib (150 mg)	488	9	7
		vs. placebo	243	<1	5
ISEL, 2005 [13]	III	Gefitinib (250 mg)	1129	8	5
		vs. placebo	563	1	5

G/C, gemcitabine/cisplatin; *C/P*, carboplatin/paclitaxel; *pts*, patients

*The trial randomisation utilised two dosages of gefitinib, 250 and 500 mg, but no differences were found in terms of efficacy

ly responsive tumours containing somatic mutations of the EGFR gene. Several studies with different design have focused on the possible predictive value of EGFR mutations in NSCLC (Table 3).

Mutations in the EGFR TK domain are found mostly in subjects who respond to gefitinib, as reported in the first three studies conducted in 2004 [17–19]. The same mutations are more frequent in women, in adenocarcinoma, in non-smokers and in Japanese subjects, all with a statistical significance [17]. These were clustered in exons 18, 19 and 21 and were either small in-frame deletion (746–750, adjacent to K745: ELREA aminoacids) or heterozygous missense mutations (mainly L858R adjacent to the DFG motif in the COOH-terminal lobe in the activation loop of the kinase) around the ATP binding pocket. Pooled data from these three studies show that 25 of 31 (81%) patients with partial response or marked clinical improvement have an EGFR mutation, *vs*. none of 29 specimens from refractory patients (p=0.0001).

Several other studies investigate the role of these mutations in the treatment of NSCLC with EGFR TK inhibitors, demonstrating a statistically significant correlation between the response to gefitinib and erlotinib and the presence of the biological data, but this correspondence is not always 100% [20–28] (Table 3). In fact objective responses are also described in patients with a wild-type EGFR, although with a lower percentage, ranging from 10% to 13% [21–23].

The probability of EGFR TK inhibitor efficacy not only increases with the presence of a mutational status, which results in the principal feature linked with response, but also correlates with female gender, non-smoker status, Asiatic origin and adenocarcinoma histology [21–28]. Other characteristics also influence tumour response, such as a greater number of prior CTx lines, or a younger age, but they are reported in only a few papers [21, 22]. Furthermore, patients with EGFR mutations survive for a longer period than those without the mutations after initiation of gefitinib treatment (p=0.0053). A specific mutation (del746–750) is found to be superior to other (L858R) mutations for the prediction of response to gefitinib [23]. The same advantage in terms of time to progression (21.7 *vs*. 1.8 months; p<0.001) and survival (30.5 *vs*. 6.6 months; p<0.001) is demonstrated in the Korean study [25].

The treatment with gefitinib of only Japanese patients after a first recurrence shows 53% objective responses, a median time to progression of 5 months, and a median survival of 16 months. Thirty-nine out of 66 patients had EGFR mutations, and their response rate (82% *vs*. 11%; p<0.0001), time to progression (12.6 *vs*. 1.7 months; p<0.0001) and overall survival (20.4 *vs*. 6.9 months; p=0.0001) are higher in comparison to the wild-type patients [24]. These data once again show the higher sensitivity of the Asiatic population to EGFR-TK inhibitors.

Considering the importance of this data, the contribution of molecular alterations in EGFR to response and survival was determined in the patients treated inside the IDEAL and INTACT gefitinib trials, retrospectively. EGFR mutations were found in 14 out of 79 cases from the IDEAL studies' available tumour samples, and in 32 out of 312 cases from the INTACT trials, including amino acid substitutions, in-frame deletions clustered around the ATP binding pocket. These mutations result more frequently in adenocarcinomas than in tumours with other histologies (17% *vs*. 5%; p=0.0001), in women than men (19% *vs*. 9%; p=0.006), in non-smokers than smokers (26% *vs*. 8%; p=0.0004) and in Asians than non-Asians (19% *vs*. 11%; p=0.346). Patients whose tumours have an EGFR mutation show a better response to gefitinib, with an overall response of 46% *vs*. 10% (p=0.005). Median time to progression for mutation positive cases was longer

Table 3 EGFR gene mutations and clinical response rate to EGFR TK inhibitors

Author, year	Inhibitor	Number of pts	Responder pts (number)	Number of responder pts with EGFR gene mutation (%)	*p* value
Paez et al., 2004 [17]	Gefitinib	9	5	5 (100)	0.0027
Lynch et al., 2004 [18]	Gefitinib	16	9	8 (88)	0.001
Pao et al., 2004 [19]	Gefitinib	24	12	9 (75)	0.001
	Erlotinib	36	10	8 (80)	0.001
Kondo et al., 2005 [20]	Gefitinib	12	4	4 (100)	NA
Rosell et al., 2005 [21]	Gefitinib	34	10	7 (70)	0.0003
Taron et al., 2005 [22]	Gefitinib	68	22	16 (73)	0.0001
Mitsudomi et al., 2005 [23]	Gefitinib	59	26	24 (96)	0.0001
Takano et al., 2005 [24]	Gefitinib	66	35	32 (91)	0.0001
Han et al., 2005 [25]	Gefitinib	90	21	11 (52)	0.001
Tomizawa et al., 2005 [27]	Gefitinib	20	14	11 (79)	0.0022
Niho et al., 2005 [58]	Gefitinib	13	4	4 (100)	NA

EGFR, epidermal growth factor receptor; *TK*, tyrosine kinase; *pts*, patients; *NA*, not assessed

(116 days *vs.* 57 days), but no impact was detected on overall survival. Molecular analysis reveals that 13 of 18 EGFR mutation carriers (72%) respond to chemotherapy plus gefitinib, compared to 84 of 152 mutation-negative cases (55%), but this difference does not achieve statistical significance (p=0.2) [26].

The majority of these studies were conducted testing the activity of gefitinib, but similar data were obtained with erlotinib. In particular Eberhard et al. analysed the mutational status and its impact on patients treated in the TRIBUTE trial [28]. Tumour EGFR mutations were identified in 29 of 228 subjects (12%) and among them 17% were never smokers. The overall response rate improved in patients treated with erlotinib and CTx, who express EGFR mutations in comparison with the wild-type tumours, and both time to progression and survival are affected by EGFR mutational status. Moreover, among EGFR mutants, overall response rate is higher in the erlotinib plus CTx arm compared to CTx alone, but this does not reach statistical significance.

Finally, a recent study shows not only that EGFR mutations are statistically associated with Asian ethnicity and never-smoker status, but also that patients with EGFR exon 19 deletions have significantly longer median survival than patients with EGFR L858R mutation (34 *vs.* 8 months, p=0.01) after treatment with gefitinib or erlotinib [29].

These findings suggest that testing for EGFR mutations could effectively be not only of predictive but also of prognostic importance for patients with NSCLC, but the relevance of such testing in selecting patients for EGFR TK inhibitor therapy remains incompletely defined, as these data emerge from studies limited by unspecific end points and design [30].

Another unclarified point is related to the method of assessing mutations, which was different in several trials, in the absence of a standard method. To this end, recent investigations concern the validation of novel assessments, such as a dual technical approach: direct sequencing of polymerase chain reaction (PCR) products and PCR single-strand conformation (SSC) polymorphism (SSCP) analysis. The SSCP analysis results in more sensitive than direct sequencing of PCR products, and consists of a rapid and reliable method for the screening of EGFR kinase domain mutations [31]. Evaluations of newer techniques, like mutant-enriched PCR and DNA endonuclease SURVEYOR, are ongoing, resulting in valuable methods in collecting preliminary data [32, 33].

EGFR amplifications

The role of EGFR amplification in the relationship with NSCLC prognosis and clinical response to the treatment with small molecules like TK inhibitors is still controversial (Table 4).

Hirsch et al. first report that EGFR gene copy number correlates with EGFR protein expression, but not with prognosis [34]. Considering these data, the relationship among EGFR gene copy number, EGFR protein expression and EGFR mutations (evaluated by fluorescence *in situ* hybridisation (FISH) and immunohistochemistry) was further investigated in 102 NSCLC patients, treated with gefitinib. EGFR gene amplification and high protein expression are significantly associated with a better clinical response, disease control rate, time to progression and survival, while EGFR mutations correlate with clinical response and time to progression. In multivariate analysis only the EGFR amplification is significantly associated with a better survival [35]. The same statistically significant benefits in terms of response rate and time to progression are reported in a Japanese study that also observed more frequent EGFR gene amplification in patients with EGFR mutations than in patients with wild-type EGFR (p=0.014) [24].

Several other studies suggest the importance of the amplification for the activation of the EGFR signalling pathway, particularly when both gene amplification and EGFR mutation are found in the same tumour, reaching a response rate to gefitinib of 100% in the trial conducted by Taron et al. [20, 22, 24, 26]. Nevertheless the latter also showed a response rate of 45% in the patients with amplified EGFR in contrast with 89% of patients with EGFR mutations (p=0.02). From these data it is difficult to determine the extent to which EGFR amplification in the absence of mutations is predictive of response [22].

Finally, the retrospective study on the tumour specimens collected in the IDEAL and the INTACT trials included not only the EGFR mutation analysis but also the EGFR gene amplification analysis by PCR. Amplification of EGFR locus was observed in 7 of 90 IDEAL cases (8%) and in 33 of 453 INTACT cases (7%). There is no significant increase in the prevalence of EGFR amplification in cases with clinical features that are characteristic of strong responses to gefitinib. In tumours analysed for both mutations and amplification of EGFR, 6 of 10 patients (60%) with either genetic abnormalities had a response to gefitinib, compared with 5 of 52 patients (10%) with neither amplification nor mutations (p=0.0011), supporting the hypothesis that genetic lesions in EGFR are critical in defining TK inhibitors susceptible subtypes of NSCLC [26].

On the contrary, Endo et al. surprisingly find that EGFR amplification does not correlate with EGFR mutation status, either with any of the clinico-pathological features or with overall survival. The authors also show a high sensitivity of TaqMan PCR to detect the mutation status [36].

On the basis of these contradictory results it is actually very difficult to give an exact definition of the role of EGFR amplification as prognostic and predictive factors, and even more difficult to clearly understand the relationship between EGFR mutations and EGFR gene amplification [37].

Table 4 EGFR amplification and clinical efficacy of gefitinib

Author, year	Number of pts	Number of pts with EGFR amplification	Response rate (%) in pts with FISH+ *vs.* FISH–	Time to progression (months) in pts with FISH+ *vs.* FISH–	Overall survival (months) in pts with FISH+ *vs.* FISH–
Cappuzzo et al., 2005 [35]	102	33	36 *vs.* 3, p=0.001	9.0 *vs.* 2.5, p=0.001	18.7 *vs.* 7, p=0.03
Kondo et al., 2005 [20]	12	2	NA	NA	NA
Takano et al., 2005 [24]	66	29	72 *vs.* 38, p=0.005	9.4 *vs.* 2.6, p=0.038	NA
Taron et al., 2005 [22]	–	28	45 (FISH – NA)	NA	NA
Endo et al., 2005 [36]	27	4	NA	NA	No correlation
Bell et al., 2005 [26]	90	7	29 *vs.* 15, NA	NA	NA

Pts, patients; *EGFR*, epidermal growth factor receptor; *FISH*, fluorescence in situ hybridisation

EGFR downstream signalling

While the mutational status seems to be important in determining the clinical response to EGFR TK-inhibitors, recent evidence suggests that genes implicated in the downstream of EGFR signalling are related not only to cancer pathogenesis but also to the clinical response to these molecular drugs.

The EGFR-dependent activation of the Ras/Raf/MAPK and PI3/Akt pathways may be involved in the sensitivity to EGFR TK inhibitors because of their role in cell proliferation and survival.

In this field, the presence of Akt in its active phosphorylated status (p-Akt) is associated not only with a better response to gefitinib (p=0.003), disease control rate (p<0.001) and time to progression (p=0.004), but also with female gender (p<0.001), never-smoking status (p=0.004) and bronchioloalveolar carcinoma histology (p=0.034). No correlation is found with p-MAPK [38].

On the contrary, no significant correlation between EGFR mutation and expression of p-Akt or p-Erk emerges in another study [25].

ErbB signalling pathways also include downstream GTPases encoded by Ras genes. K-ras mutations occur in 10%–30% of NSCLC cases, especially in codons 12 and 13 encoded by exon 2, showing a strong association with smoking history and with poor prognosis [39]. Recent studies demonstrate that EGFR and K-ras mutations are mutually exclusive [40]. In fact, when the EGFR signal pathway is activated by the genetic alteration of EGFR, the mutation of Ras may not be necessary for the signal transduction, as Ras is also one of the downstream molecules in this pathway [20, 27]. The Italian investigation into the relationship between EGFR and K-ras mutations in 860 NSCLC patients reports this data: all of the tumours affected by mutated EGFR are found to be negative for K-ras mutations, whereas tumours negative for EGFR mutations show a K-ras mutation in 32% of cases (p=0.000001) [31].

In the TRIBUTE trial, K-ras mutations are detected in 21% of tumours, and are associated with significantly decreased time to progression and survival in patients treated with erlotinib and chemotherapy [28]. Mutations in K-ras are found more frequently in patients who develop disease progression with either gefitinib or erlotinib [41]. Braf mutation is uncommon both in Caucasian and in Japanese NSCLC patients [42, 43], and at the moment no data is available on its role.

From these studies in particular, a molecular aetiology- and pathogenesis-related difference emerges: NSCLC arising in smokers seem to have a dissimilar spectrum of molecular alterations than those seen in non-smokers, as the basis of their opposite prognosis and responsiveness to EGFR TK inhibitors. In fact the presence of a K-ras mutation, very frequent in smokers, could likely constitute a useful marker for selecting those patients who will not benefit from anti-EGFR therapy.

The relationship between somatic mutations of EGFR and K-ras genes and DNA methylation of tumour suppressor genes has been recently investigated, and a specific interaction of genetic and epigenetic changes in the tumorigenesis of NSCLC is identified [44]. Nowadays the microarray analysis could represent a potentially useful approach to better define the complexity of NSCLC carcinogenesis, including the molecular pathways that could be associated with smoking habits and gender [45].

Finally, abnormal PI3K/Akt and Ras/Erk pathways seem to be correlated with tumour insensitivity to receptor blockade, and considering this information, the combination treatment of TK inhibitors and inhibitors of the PI3K/Akt and Ras/Erk pathways may provide a successful strategy [46].

Other members of the EGFR family

Because of the complex crosstalk among the EGFR family members, the TK inhibitor sensitivity could be related

not only to the EGFR presence, but also to the influence of HER2 and HER3.

Preclinical data indicate that HER2, a member of the EGFR family, could enhance TK inhibitor sensitivity. EGFR family members, in fact, exist as monomers and the monomeric receptors dimerise and become functionally active after binding to the appropriate ligand; HER2 represents the preferred EGFR partner.

Independent of the method for EGFR assessment, increased copy numbers of the HER2 gene enhance sensitivity to gefitinib in patients with EGFR-positive tumours, while mutations in the TK domain of the HER2 gene seem to be infrequent and not clinically relevant. Patients with HER2 high copy number (22.8%), detected by FISH, show significantly better objective responses, disease control rate and time to progression compared with patients with HER2 FISH-negative. Also, HER2 protein expression, tested by immunohistochemistry, shows positive results in only 7% of patients, and no HER2 mutations in exon 20 are found. HER2 gene gain is significantly associated with EGFR gene gain (p=0.004) and with EGFR gene mutations (p=0.003). Patients with HER2 FISH-positive tumours with an increased expression of EGFR protein, gene gain or EGFR mutations have a significantly better clinical outcome than patients negative for both receptors. On the contrary, in the absence of EGFR mutations, the outcome of HER2-positive patients is the same as the outcome of patients negative for both receptors, which is the worst scenario for all the clinical end points [47].

These data suggest a strong rationale to explore the association of TK inhibitors and anti-HER2 agents, emphasising their probable synergistic effects.

The correlation between EGFR and HER2 was also studied, with controversial results. Neither mutation nor expression of EGFR and HER2 are significantly related to the prognosis. However, the number of HER2 mutated patients was too small to accurately determine prognostic association. Despite this limitation, an interesting relationship emerges: EGFR overexpression results more frequently in tumours with EGFR mutations (p=0.0059) [48].

A series of patients treated with gefitinib were evaluated for HER3 genomic gain by FISH. HER3 FISH-positive pattern is significantly associated with female gender and never smoking history, but this gene does not emerge as a marker of response or resistance to TK inhibitors.

These data suggest a possible association between the activation of these pathways and the presence of EGFR mutations [49].

Acquired resistance to EGFR TK inhibitors

A substantial proportion of NSCLC patients treated with gefitinib and erlotinib will ultimately develop a disease relapse. The mechanism of such acquired drug resistance remains practically unknown.

First, a secondary mutation in exon 20, leading to a substitution of methionine for threonine at position 790 (T790M) in the kinase domain, is reported in 2 of 5 patients with acquired resistance to gefitinib or erlotinib [50].

An interesting case report describes the same mutation associated with gefitinib resistance [51]: a 71-year-old smoker, with lung adenocarcinoma resistant to CTx, had a complete remission after treatment with gefitinib. After 24 months of therapy, the tumour recurred. Exons 18–21 of the EGFR gene were sequenced from DNA isolated from both the original diagnostic biopsy and the specimen obtained at the relapse: while a small deletion mutation (delL747-S752) was detected in both the biopsies, the presence of a second point mutation, resulting in threonine-to-methionine amino acid change at position 790 of EGFR (T790M), was detected only in the relapse specimen. The delL747-S752 belongs to the mutations described above associated with gefitinib responsiveness, and this report confirms these data. The appearance of a second mutation represents a mechanism of resistance: in fact the authors demonstrate that the insertion of T790M into test cells renders them resistant to gefitinib *in vitro*. They also find that when test cells transfected with both mutations are treated with other EGFR inhibitors, such as AG1478, cetuximab, erlotinib or CL-387,785, no objective response is obtained using the first three agents, while the fourth is effective. The sensitivity of the delL747-S752+T790M construct to the anilinoquinazoline CL-387,785 might be explained either by its altered binding to the kinase domain or its covalent binding to EGFR. These data support clinical investigations of compounds similar to CL-387,785 in order to identify optimal treatment strategy for patients with resistance to EGFR inhibitor therapy caused by T790M or other mutations [52]. A phase I dose escalation study was conducted in Japanese patients in order to test the efficacy of EKB-569, an oral EGFR inhibitor.

Preliminary data were recently published that show that this agent has clinical activity in 2 patients with advanced NSCLC with EGFR mutations and acquired gefitinib resistance [53]. Finally, a recent retrospective analysis of IDEAL trial specimens does not find any T790M mutations in the tumours analysed [26].

Preclinical studies are ongoing not only to investigate the biological mechanisms of resistance to EGFR TK inhibitors in greater depth, but also to identify new agents potentially able to overcome these phenomena [54, 55].

Timing of treatment with EGFR TK inhibitors

Several case reports are published in which EGFR TK inhibitors are utilised upfront in patients with EGFR mutations, and who are not eligible for a standard cytotoxic

CTx [56]. These reports raise the question about the possible role of these agents as first-line therapy, and clinical studies were conducted.

A phase II study of gefitinib as first-line treatment in 36 never-smoking patients was conducted in South Korea, obtaining a response rate of 69% [57].

Another recent phase II study investigated the role of gefitinib as first-line treatment in 40 eligible patients with advanced NSCLC. In case of absence of tumour reduction within 4 weeks or partial response within 8 weeks, the treatment was interrupted, shifting to a platinum-based CTx. The response rate was 30%, median survival time was 13.9 months and 4 patients developed a grade 5 interstitial lung disease (10%). The response rate was statistically correlated with adenocarcinoma histology (p=0.0048), female gender (p=0.0050) and non-smoking status (p=0.0048). Tumour samples were available for 13 patients (4 partial response, 6 stable disease and 3 progressive disease): EGFR mutations were detected in 4 responder patients. The response rate of the second line treatment was 30%, and it does not seem to be adversely affected by pre-treatment with gefitinib [58].

Nowadays a key question about the use of EGFR TK inhibitors in NSCLC is the rationale of their employment as first-line treatment of advanced disease as well as neoadjuvant or adjuvant treatment in those patients whose tumours have EGFR mutations. In fact, the real impact of EGFR mutations in making a therapeutic decision has not been well defined because this target included the majority but not all responders, as has been revealed by the above-mentioned studies.

To date, no conclusive data are available from large, prospective clinical trials evaluating these agents in earlier treatment setting, and the availability of such biologic drugs approved for the treatment of recurrent disease selected patients.

Conclusions and open issues

During recent years, the development of targeted anticancer therapy has become more promising than the optimisation of medical treatment with conventional anticancer agents. In NSCLC, the targeting of EGFR TK, by the small molecules gefitinib and erlotinib, has led to more options in the management of patients with advanced disease. The two drugs followed the same development procedure and, in spite of the large number of studies performed to date, further research is still warranted to define the ultimate clinical role of these agents.

In the field of prognostic and predictive factors that will aid a clinically useful patient selection, it is necessary to initiate large and well designed clinical trials with EGFR TK inhibitors in different regions of the world, and to focus on patient subsets characterised by specific clinical and histopathological features.

Furthermore, our understanding of the molecular profile in a tumour that may predict clinical response remains naïve, and as the molecular heterogeneity of NSCLC becomes more apparent, it is necessary to direct the research to identify the biomarkers that will indicate which patients are most likely to experience therapeutic benefit. To this end it is essential to incorporate the EGFR mutational profile and the characterisation of the genes involved in the EGFR signalling, into specifically designed clinical trials. This also means the urgent need for a careful standardisation of laboratory methods for molecular marker characterisation.

Preclinical studies continue to be important, in particular to better define the potential role of the association between different EGFR family members as well as to elucidate the mechanisms of resistance to EGFR inhibitors (and the possible strategies to overcome it).

At the same time, efforts should be directed to a better understanding of useful strategies for associating these agents with conventional CTx, also taking into account that no exhaustive data is available on the possible effect of CTx on EGFR expression or mutations.

Future research should also involve the identification of the phase of disease in which these agents can be best employed (such as the neoadjuvant and the adjuvant setting), together with the validation of new surrogate markers of clinical response to these drugs. In fact, the effect of molecular agents on tumour cells seems to be cytostatic rather than cytotoxic so that the standard methodology utilised for the assessment of efficacy in clinical cancer research may not be the best one to test for the efficacy evaluation of the targeted therapy.

In conclusion, EGFR TK inhibitors are promising anticancer agents, but all the questions mentioned above have not been completely answered by the relatively high number of studies conducted to date (Table 5). Hence, designing spe-

Table 5 Open issues about EGFR TK inhibitors employment in the treatment of NSCLC

Identification and validation of clinical and biological prognostic/predictive factors
Definition of standard methods for molecular target characterisation
Importance of the relationship among different EGFR family members
Better comprehension of the mechanisms of resistance and their possible treatment
Role of the association with conventional chemotherapy
Utilisation as neoadjuvant, adjuvant and first-line treatment
Specifically designed clinical trials including new surrogate end points, conducted in selected patient populations

cific clinical trials to further investigate the activity of this drug class and optimise their use in prospectively defined patient populations are critical challenges to the final success of this therapeutic approach. Only in this way, within the next few years, will we find out whether we can make a real paradigm shift in the treatment of NSCLC by translating all the basic scientific progress into clinical practice.

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