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Clinical Development of Epidermal Growth Factor Receptor (EGFR) Tyrosine Kinase Inhibitors: What Lessons Have We Learned?

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10.1 Introduction

The epidermal growth factor receptor (EGFR) was selected as a strategic target for anticancer drug development almost two decades ago. This was based on evidence of receptor over-expression in human cancer and association with worse prognosis. Therapeutic strategies were developed and showed preclinical evidence of antitumor effects in animal models of EGFR-driven tumors. The fundamental process leading to EGFR dysregulation in human cancer were not known at that time. These agents were among the first class of targeted agents to enter the clinic at a time when the need to change the clinical development process use for cytotoxic agents to accommodate this new class of drugs was starting to be discussed. Two areas were of major interest. One was to base dose selection in pharmacodynamic endpoints rather than toxicity-based criteria. The second was to elucidate which patients are more likely to respond to these agents. Over the last few years this has been an important area of research. We have learned that while pharmacodynamic endpoints are ideal, the lack of robust and well validated analytical methods may lead to the wrong dose selection. In addition, while the average patient may benefit from these treatments, it is now clear that patients with genetic dysregulation of the EGFR by either mutations or amplifications or both are the best candidates for these treatments. It is not clear, however, how to learn about these predictors of response at earlier stages in the clinical development so that enrichment strategies can be implemented.

10.2 The HER Family of Receptors

The EGFR (HER1) is a member of the HER family of membrane receptors (HER1 through 4). The other members are HER2 (also termed ErbB2 or HER2/*neu*), HER3 (also termed ErbB3), and HER4 (also termed ErbB4). These receptors share

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the same molecular structure with an extracellular, cysteine-rich ligand-binding domain, a single alpha-helix transmembrane domain, and an intracellular domain with tyrosine kinase (TK) activity in the carboxy-terminal tail (excepting the HER3) (1). The TK domains of HER2 and HER4 show an 80 percent homology to that of the EGFR (2). Epidermal growth factor (EGF), transforming growth factor α (TGFα), and amphiregulin bind exclusively to the EGFR, whereas betacellulin and epiregulin bind both the EGFR and HER4. Ligand binding induces EGFR homodimerization, as well as heterodimerization with other types of HER proteins (3, 4). HER2 does not bind to any known ligand, but it is the preferred heterodimerization partner for EGFR after ligand-induced activation (5). EGFR/EGFR homodimers are unstable, whereas EGFR/HER2 heterodimers are stable, and recycle more rapidly to the cell surface (6). EGFR dimerization induces TK catalytic activity, which leads to the autophosphorylation in one or more of the five tyrosine residues in the carboxy-terminal tail, producing phosphotyrosine sites (Y992, Y1068, Y1086, Y1448, and Y1173) where adaptor and docking molecules ultimately bind (7). EGFR intracellular signalling is mainly mediated through two interrelated downstream pathways – the Ras-Raf-mitogen-activated protein kinases (MAPK, also known as extra-cytoplasmatic regulated kinases, ERK1 and ERK2), and the phosphatidylinositol 3-kinase (PI3K)/Akt pathways (8, 9). ERKs regulate the transcription of molecules involved in cell proliferation, transformation, and metastasis development (10), whereas the Akt pathway is more relevant in cell survival processes (11). An alternative route of EGFR-mediated transduction of extracellular signals is via the stress-activated protein kinase pathway that involves protein kinase C (PKC), although the basis of this regulation remains obscure. The finding that PKC has a role in EGFR transactivation and ERK regulation further complicates this regulatory mechanism (12). EGFR signaling ultimately causes increased proliferation (13), angiogenesis (14), metastasis (15), and decreased apoptosis (16). Under physiological conditions ligand binding is required to activate EGFR; however, in tumor cells there are additional mechanisms of EGFR activation. First, receptor over-expression leading to ligand-independent dimerization is commonly found in many different solid human tumors (17). Second, autocrine production of ligands (such as TGF() by tumor cells has been linked to receptor over-expression, and may represent an efficient mechanism of EGFR-driven growth (18).

10.3 Early Days Rationale to Target the EGFR

The rationale to target the EGFR for cancer therapeutics is elegantly described by Dr. John Mendelshon during his David A. Karnosky Award Lecture in 2002 (19). As described in that paper, the key concepts that led to strategies targeting the EGFR were the notion that growth factor receptors, in general, were attractive therapeutic targets, particularly for monoclonal. The EGFR was selected among many growth factor receptors because the EGFR over-expression correlates with a worse clinical outcome

in several cancers including non-small-cell lung cancer (NSCLC), and tumors of the prostate, breast, stomach, colon, ovary, and head and neck, further supporting their role in tumorigenesis (17, 20, 21). It is estimated that between 40 percent and 80 percent of NSCLC over-express EGFR, and 20 to 30 percent over-express HER2 (22-24). The pivotal role that the EGFR plays as a sensor of the extracellular environment and the maintenance of cellular homeostasis makes it an *a priori* ideal candidate for a cell in transformation to exploit in order to acquire advantageous features such as freedom of movement, nutrient independence, and immortality. The EGFR was proposed as a rational target for drug development more than 20 years ago (25, 26).

10.4 Strategies to Inhibit the EGFR

Numerous classes of drugs that target the EGFR are under development, and over the last few years, an increasing number of compounds directed against the EGFR have entered clinical development and are currently in clinical trials. Two strategies have been more extensively explored in the clinic. One is when small molecules that compete with adenosine triphosphate (ATP) for binding to the receptor's kinase pocket, thus blocking receptor activation, also known as TK inhibitors (TKI). The other is when monoclonal antibodies (MAbs) are directed against the external domain of the receptor. In this article we will focus on small molecules – TKI.

TKIs compete with adenosine triphosphate (ATP) for binding to the receptor's kinase pocket, thus blocking receptor activation. A large number of TKIs are currently being evaluated. They can be classified according to their selectivity (specific agents with HER1-selective activity, as opposed to non-specific agents that target several members of the HER-family or other receptors), according to the reversibility of their interaction with their target (reversible or irreversible inhibitors) (Table 10-1). Two have received regulatory approval for use in NSCLC patients – gefitinib and erlotinib – and will be the focus of this paper.

10.5 Gefitinib

Gefitinib (Iressa, ZD1839, AstraZeneca) is an orally active, low molecular weight, synthetic quinazoline (27). Gefitinib reversibly and selectively targets the EGFR and blocks signal transduction processes implicated in the proliferation and

Table 10-1 Small molecules targeted to the EGFR.IC_{50} values represent substrate phosphorylation assays

Drug	Type	EGFR IC_{50} (μM)	HER-2 IC_{50} (μM)	Phase of development
Gefitinib (Iressa™, ZD1839)	Selective, reversible	0.02	3.7	Approved
Erlotinib (Tarceva™, OSI-774)	Selective, reversible	0.02	3.5	Approved
EKB-569	Selective, irreversible	0.04	1.2	III
Lapatinib (GW2016)	Bifunctional, reversible	0.01	0.009	Approved

Table 10-2 Results of trials of gefitinib in the first-line treatment of non-small-cell lung cancer

Chemotherapy	Biologic agent	Response rate (%)	Median survival (months)	1-year survival (%)	Ref
Gemcitabine and cisplatin	Placebo	44·8	11·1	45	(36)
	Gefitinib 250 mg	50·1	9·9	42	
	Gefitinib 500 mg	49·7	9·9	44	
Paclitaxel and carboplatin	Placebo	33·6	9·9	42	(37)
	Gefitinib 250 mg	35·0	9·8	42	
	Gefitinib 500 mg	32·1	8·7	38	

survival of cancer cells with minimal activity against other tyrosine kinases and serine/threonine kinases. Gefitinib prevents autophosphorilation of EGFR, resulting in the inhibition of downstream signalling pathways (28-30).

Phase I clinical trials of gefitinib showed a favorable toxicity profile, mostly consisting of skin toxicity and diarrhea. DLTs were observed at doses well above that at which antitumor activity was seen (31-33). Two Phase II studies have evaluated the clinical activity of gefitinib at two dose levels (250 and 500 mg) in patients with NSCLC that had failed at least one (210 patients) and at least two (216 patients) chemotherapy regimens for advanced disease, documenting response rates of 18.7 percent and 10.6 percent, respectively (34, 35). In these studies, a higher dose did not improve response rate and caused an increase in toxicity. Improvement in disease-related symptoms was significant in both trials. These results led to the regulatory approval of gefitinib (250 mg/d) as monotherapy treatment for patients with locally advanced or metastatic NSCLC refractory to platinum-based and docetaxel chemotherapy in the United States and Japan, among others. However, the addition of gefitinib to standard chemotherapy has failed to induce an improvement in response or survival in chemo-naïve NSCLC patients. Two placebo-controlled, double-blinded, Phase III randomized trials evaluating chemotherapy (either gemcitabine-cisplatin or paclitaxel-cisplatin) plus either gefitinib (250-500 mg) or placebo have rendered negative results (36, 37) (Table 10-2). A placebo-controlled Phase III study investigated the effect on survival of gefitinib as second-line or third-line treatment in 1,692 patients with locally advanced or metastatic non-small-cell lung cancer (38). The primary endpoint was survival in the overall population of patients and those with adenocarcinoma. Pre-planned subgroup analyses showed longer survival in the gefitinib group than the placebo group for never-smokers (n=375; 0.67 [0.49 - 0.92], p=0.012; median survival 8.9 vs 6.1 months) and patients of Asian origin (n=342; 0.66 [0.48 - 0.91], p=0.01; median survival 9.5 vs 5.5 months), but treatment with gefitinib was not associated with significant improvement in survival in either co-primary endpoint.

10.6 Erlotinib

Erlotinib (Tarceva, OSI-774, OSI Pharmaceuticals) is a quinazoline derivative, which reversibly inhibits the kinase activity of EGFR. It has shown in vitro and in vivo activity in preclinical trials in multiple human cancer cell lines, including

ovarian, head and neck, and non-small-cell lung carcinoma (39, 40). Erlotinib has been evaluated in several Phase I studies using different doses and schedules, including weekly administration for three weeks every four weeks, and a continuous daily dosing (41, 42). The schedule that was ultimately chosen for further evaluation consists of the daily administration of 150 mg orally, with higher doses resulting in dose-limiting diarrhea and cutaneous acneiform rash (41). The cutaneous toxicity was dose-dependent, affected the face and upper trunk areas, appeared at the end of the first week of dosing and progressively recovered even in patients who continue taking the same dose of erlotinib. Other toxicities were mild to moderate and consisted of nausea and vomiting, elevation in bilirubin, headaches, and mucositis. The preliminary results of several disease-directed studies have been presented. Erlotinib has demonstrated clinical activity as a single agent in patients with NSCLC, ovarian cancer, and SCCHN (43-45). A combined analysis of the data of these Phase II studies showed that patients who developed a rash of any grade had a statistically significant longer median survival (46).

Data from two Phase III clinical trials in patients with non-small-cell lung cancer comparing standard chemotherapy regimens [cisplatin plus gemcitabine (47), and carboplatin plus paclitaxel (48)] with or without erlotinib showed that this approach failed to demonstrate a response or survival advantage. However, in a trial that randomized pretreated NSCLC patients, 2:1 to erlotinib:placebo subjects receiving the study drug survived 6.7 months compared with 4.7 months of those taking placebo ($p < 0.001$), and has been the first EGFR-targeted therapy to receive regulatory approval on the basis of prolongation of survival (49).

10.7 Insights Gained in the Role of EGFR in Cancer

In parallel to the clinical trials mentioned above and as the number of patients treated with these agents increased, a number of groups started to rationally seek factors that may be linked to the activity of the compounds. The first evidence came from clinical observations. It was known that female patients, patients of Asian origin, never -smokers and those with an adenocarcinoma type of NSCLC were the subgroups more likely to benefit from these agents. Subsequent molecular studies did not reveal the cause of this observation. This includes the link between receptor amplification and response to these agents, as well as the discovery of activating mutations of the *egfr* gene.

Abnormalities in *egfr* copy number are frequent in cancer. In a report that investigated *egfr* and EGFR expression (by fluorescent in situ hybridization [FISH] and immunohistochemistry [IHC], respectively) in 183 NSCLC patients, trisomy, polysomy and gene amplification were observed in 40 percent, 13 percent and 9 percent of the cases, respectively (50). EGFR over-expression was observed in 62 percent of the cases and correlated with increased gene copy number. Increased EGFR gene copy number detected by FISH is associated with improved survival after gefitinib therapy in patients with NSCLC (51). In this report,

amplification or high polysomy of the *egfr* (documented in 33 of 102 patients) and high protein expression (observed in 58 of 98 patients) were significantly associated with better response (36% versus 3%, mean difference = 34%, 95% CI = 16.6 to 50.3; $P < 0.001$), disease control rate (67% versus 26%, mean difference = 40.6%, 95% CI = 21.5 to 59.7; $P < 0.001$), time to progression (9.0 versus 2.5 months, mean difference = 6.5 months, 95% CI = 2.8 to 10.3; $P < 0.001$), and survival (18.7 versus 7.0 months, mean difference = 11.7 months, 95% CI = 2.1 to 21.4; $P = 0.03$). Similar results regarding the correlation between *egfr* copy number and outcome were observed in a cohort of subjects with advanced bronchioalveolar carcinoma (BAC) (52). These two reports suggest FISH can be used to assess survival potential in patients treated with EGFR TKIs. In the latter subset of lung cancer subjects, no association was found between HER2 gene copy number and response or survival. Interestingly, in another report, increased HER2 copy number was also a solid marker of response to gefitinib therapy in a broader lung cancer population (53). Patients with HER2 FISH-positive tumors displayed increased expression of EGFR protein and gene gain. These findings highlight the relevance of the interplay between the HER family of receptors in the pathogenesis of cancer. In the univariate analysis of the NSCLC patients receiving erlotinib or placebo in the pivotal trial, survival was longer in the erlotinib group than in the placebo group when there was high EGFR expression (hazard ratio, 0.68; $P = 0.02$), or there was a high number of copies of *egfr* (hazard ratio, 0.44; $P = 0.008$) (54), but these correlations were not evident in the multivariate analysis.

Recent data have shown that mutations in the ATP-binding site of the *egfr* gene predict sensitivity of NSCLC patients to gefitinib (55, 56). In the report by Lynch, et al mutations were identified in the tyrosine kinase domain of the *egfr* gene in eight out of nine patients with gefitinib-responsive lung cancer, as compared with none of the seven patients with no response ($P<0.001$) (55). In the report by Paez, et al somatic mutations of the *egfr* gene were found in 15 of 58 unselected tumors from Japan and one of 61 from the United States (56). This phenomena was not agent- or family-specific, as it has been also documented in NSCLC patients treated with erlotinib (57), and in cell lines treated with the bifunctional (EGFR plus VEGFR2/KDR) inhibitor ZD6474 (58).

Mutations were either in-frame deletions or amino acid substitutions clustered around the ATP-binding pocket of the tyrosine kinase domain of EGFR. Remarkably, many of these deletions overlapped, sharing the deletion of four amino acids within exon 19. Other tumors had amino acid substitutions within exon 21, being particularly frequent and consistent in several reports the change from leucine to arginine at codon 858 (L858R). All mutations were heterozygous, and identical mutations were observed in multiple patients, suggesting an additive-specific gain of function. Matched normal tissue from available patients showed only the wild-type sequence, indicating that the mutations had arisen somatically during tumor formation. To further support the pathogenic role of mutations in determining the response of NSCLC to EGFR TKIs there are already reported cases where secondary mutations reverse an initial sensitivity to those agents (59).

The location of the mutations influences the sensitivity to EGFR inhibition. Gefitinib was more effective in patients with the deletion type of mutations than in patients with other mutations such as L858R (60). The response rate of patients with an exon 19 deletion and L858R were 84 percent and 71 percent, respectively, but only about half of the subjects bearing G719X had an objective response to gefitinib. In addition, patients with exon 19 deletions had a longer median survival after erlotinib or gefitinib than those with L858R (34 vs 8 months, respectively; P = 0.01) (61). In an analysis of erlotinib sensitivity using mutant constructs the order of sensitivity was exon 19 deletion = L858R > G719X > exon 20 insertion = wild-type, which is similar to the clinical observations so far (62).

In those initial, retrospective and non-consecutive analyses mutations were more prevalent in female patients with adenocarcinoma histology, and in Asian ethnic backgrounds. The report by Kosaka, et al confirmed in a systematic manner what had been described in the anecdotal initial series of NSCLC patients (63). *Egfr* mutations were not related to age or clinical stage, but there was a strong positive correlation between female gender, non-smoking status, adenocarcinoma subtype, and high degree of differentiation to mutation presence. Across all reports, independently of ethnic origin, *egfr* mutations appear almost exclusively in adenocarcinomas. It is relevant to note that as opposed to Western patterns, adenocarcinoma accounts for the majority of the NSCLC cases in Japan – as much as 70 percent in a series of resected cases (64). The actual difference in incidence of mutations between Japanese and American populations may, in part, arise from different ethiopathogenic factors mostly evidenced by profoundly dissimilar tobacco consumption, especially in women. Spontaneous mutation occurrence in predisposed histologic glandular cell subtypes, as opposed to carcinogen-induced in epithelial cells, may be behind these differential patterns.

In one of the first reports to gain further insight on the mechanistic basis of this observation, cell lines were transfected with such mutations, and mutant strains showed equivalent sensitivity to gefitinib concentrations 10-fold lower than parental cell lines (55). Differences in EGFR phosphorylation were noted and, as in transfection-induced mutated cell lines EGFR Tyr1068 phosphorylation, was more intense and also had a longer duration. These results may indicate that the mutations lower the threshold of efficacy for TKIs and thus render the EGFR susceptible to lower (clinically achievable) drug concentrations, which are suboptimal to efficaciously inhibit the receptor in the patients bearing the wild-type phenotype. As mentioned bellow, this may have explained the results of some of the clinical trials. Several reports indicate that the occurrence of EGFR mutations is an early event in carcinogenesis. Particularly, a study that analyzed mutation-positive and -negative cancers and normal adjacent mucosa showed that *egfr* mutations identical to the tumors were detected in the normal respiratory epithelium in 9 of 21 (43 percent) patients with mutant adenocarcinomas, but in none of the 16 patients without tumor mutations (65). The finding of mutations being more frequent in normal epithelium within tumor (43 percent) than in adjacent sites (24 percent) suggests a localized field effect phenomenon. In a small report in Japanese patients, *egfr* mutations were found in 12 of 19 (63 percent) of brain metastases of patients with NSCLC (66).

The same types of mutations were found in those where both primary and metastatic tissue were available, suggesting that mutation occurrence precedes systemic spread and supporting an early appearance.

A seminal report generated transgenic mice with inducible expression in type II pneumocytes of two common *egfr* mutants seen in human lung cancer (67). Both transgenic lines developed lung adenocarcinoma after sustained *egfr* mutant expression, confirming their oncogenic potential. Importantly, maintenance of these lung tumors was dependent on continued expression of the EGFR mutants and treatment with small molecule inhibitors (erlotinib or HKI-272), as well as prolonged treatment with a humanized anti-hEGFR antibody (cetuximab) which led to dramatic tumor regression. However, the pathogenic role of these mutations and its impact in downstream pathways is not completely understood. A report by Sordella, et al has shed some light in this issue, as it analyzed the differences in EGFR phosphorylation patterns in the five possible sites of the intracellular domain of the EGFR comparing mutated and wild-type NSCLC cell lines (68). Y1045 and Y1173 showed no differences, Y992 and Y1068 were more activated in mutated vs. wild-type, and Y845 was more activated in missense mutations vs. wild-type or deletion mutations. ERK status was equal in mutated vs. wild-type cell lines, probably because this signal is usually transduced via Y1173 to ras and then ERK. Phosphorylation of both Akt and STAT5 was higher in mutated vs. wild-type, as they are linked to Y992 and Y1068. These results suggest that 1) the downstream will ultimately depend on the mutation type, and 2) Akt status has questionable predictive value per se, as it fluctuates depending on the type of mutation phenotype present. It would be more informative to determine the actual subtype of EGFR phosphorylation, instead, to put into perspective the downstream scenario. In concordance to the prior data, Conde, et al determined in an analysis of the genetic and histological features of NSCLC patients that the mammalian target of rapamycin (mTOR) pathway was significantly more activated in both *egfr* and *Kras* mutants than in their wild-type counterparts (69). EGFR mutations tended to be associated with increased numbers of CA repeats and increased *egfr* gene copy numbers, but not with EGFR and caveolin-1 mRNA over-expression (70). In summary, it is increasingly evident that *egfr* mutations are oncogenic, appear early in tumorigenesis, are associated with specific signalling signatures, and induce a phenomenon of oncogene addiction that render the strains bearing them particularly sensitive to EGFR targeted therapies.

The initial reports were retrospective and, therefore, could not address the prevalence of *egfr* mutations in the general population of cancer patients. Pao and Miller reviewed this and the results are summarized in Table 10-3 (71). A consecutive series of 277 Japanese patients with NSCLC has shown a prevalence of mutations of 40 percent that were associated with female and non-smoker status, and adenocarcinoma subtype (63). A relevant aspect of this report is that *egfr* mutations were never found along with *Kras* mutations, and were more prevalent in non-smokers. In the trial that compared erlotinib with a placebo 177 samples were analyzed for *egfr* mutations, and a mutation incidence of 22 percent was documented (54). Finally, in a recently reported analysis of 860 consecutive NSCLC Italian patients

Table 10-3 Incidence of *egfr* mutations in various subgroups of NSCLC (71)

Characteristic	No. of tumors evaluated	No. of tumors with EGFR mutation	Positive for EGFR mutation (%)
Never-smokers	181	92	50.8
Smokers	434	39	9.0
Women	216	81	37.5
Men	422	55	13.0
Adenocarcinoma	453	142	31.3
Non-adenocarcinoma	306	7	2.3
East Asian	419	122	29.1
Non–East Asian	340	27	7.9
United States	262	25	9.5
Total	759	149	19.6

a global *egfr* mutation incidence of 4.5 percent was found (72). No mutations in 454 squamous carcinomas and 31 large cell carcinomas investigated were documented, and 39 were found in the series of 375 adenocarcinomas. Again e*gfr* mutations and *Kras* mutations were mutually exclusive. Bearing in mind that *Kras* serves as a downstream mediator for EGFR, the authors of the Italian report speculate that the mutually exclusive presence of *egfr* and *Kras* mutations may respond to an evolutionary paradigm where activating mutations in *egfr* are redundant if a mutation in *Kras* is already present (and vice versa). This may also help explain the striking inverse relationship of tobacco consumption and incidence of *egfr* mutations observed by this and other groups (73); it can be speculated that smoking tends to induce mutations in *Kras* that somehow prevent or make unnecessary other function-acquiring genetic changes. In addition, this downstream event seems to render EGFR-targeted therapy inefficacious, adding predictive value to its evaluation.

Few reports have addressed the independent prognostic value of *egfr* mutations. *Egfr* mutations were detected in 13 percent of 274 tumors of previously untreated patients with advanced NSCLC in the Phase III study that randomly assigned to carboplatin and paclitaxel with erlotinib or placebo. Mutation presence was associated with longer survival, irrespective of treatment ($P < .001$) (74). Whether this is directly related to the *egfr* mutation per se, or a consequence of the absence of *Kras* mutation, is unknown. Among erlotinib-treated patients, *egfr* mutations were associated with improved response rate ($P < .05$) and there was a trend toward an erlotinib benefit on time to progression ($P = .092$), but not improved survival ($P = .96$). In contrast, the Japanese report on 277 patients and the follow-up analysis of the gefitinib-treated subjects showed that whereas in patients that had not received the drug the mutational status had no significant prognostic value, the analysis of the patients that had received gefitinib revealed that the presence of the mutation had predictive value for increased survival (60, 63).

In an analysis of 90 NSCLC patients treated with gefitinib the response rate in the 17 patients harboring an *egfr* mutation was 65 percent in contrast to 13.7 percent in patients without mutation ($P < .001$) (75). Moreover, these 17 patients with EGFR mutation had significantly prolonged time to progression (21.7 v 1.8 months;

P < .001) and overall survival (30.5 v 6.6 months; P < .001) compared with the remaining 73 patients without mutation. In a recent report in 69 Korean NSCLC patients treated with gefitinib that analyzed the predictive value of several genetic and histologic parameters, there were no responders among carriers of *Kras* mutations that included two cases with concomitant *egfr* mutations (76); e*gfr* mutation presence was the only factor with predictive value in multivariate analysis. Other reports confirmed the predictive value of egfr mutations to TKIs in NSCLC patients, particularly of Asian origin (70, 77, 78). In the clinical trial that compared erlotinib with a placebo for NSCLC 325 samples were analyzed for EGFR expression and 177 samples were analyzed for *egfr* mutations (54). In contrast with other series in the multivariate analyses, adenocarcinoma (P=0.01), never having smoked (P<0.001), and expression of EGFR (P=0.03) were associated with an objective response, but survival after treatment with erlotinib was not influenced by the status of EGFR expression, the number of *egfr* copies, or *egfr* mutations (although EGFR expression and gene copy number appeared to be predictive in the univariate analysis). However, several methodological criticisms can be raised, including that mutational analysis was conducted in less than 25 percent of randomized patients, and that there is no indication that the sequencing was repeated in those positive cases (which may account for the high incidence of non-reported mutation types).

10.8 Lessons Learned

10.8.1 Dose Selection is an Important Issue

As mentioned above, while erlotinib has been approved for treatment of patients with chemotherapy-resistant NSCL, based on increased survival in a randomized clinical trial, gefitinib failed to do so. The reason underlying this discrepancy is not known. These two molecules are quite similar in mechanism of action and pharmacological properties. One possibility is the different population of patients and differences in clinical trial design. Another possibility is that gefitinib has been developed at a lower dose at which the concentration achieved is not sufficient to inhibit the wild-type receptor. Indeed, the Phase I clinical trials of gefitinib determined a maximum tolerated dose (MTD) of ~750 mg per day. However, lower doses of 250 and 500 mg were selected for initial exploratory trials. These doses were selected based on achievement of plasma levels sufficient to inhibit the receptor in the preclinical model as well as in pharmacodynamic effects in skin tissues (31-33). The studies, however, ignored the real unknown value of a plasma level, the fact that the skin and the tumor are not necessarily the same and that the methods used to determine the pharmacodynamic effects are not validated. A subsequent trial tested the efficacy of 250 versus 500 mg in NSCLC. The study concluded that the two doses were equivalent and selected the lower dose for definitive studies. The problem with this approach is that the IDEAL trials are

indeed underpower to test an equivalenct hypothesis (34, 35). As mentioned above, susceptibility to the EGFR TKI is probable and in patients with low levels of wild-type receptor to patients with amplified mutant receptor. The efficacy of the drugs against these different situations is related to drug concentrations. Thus, it is likely that at the dose recommended for clinical trials, the intratumor levels of gefitinib are only effective to inhibit the most susceptible genotypes. Because these patients are not common, in a large clinical trial the effects may get diluted. In contrast, erlotinib was developed at the MTD of 150 mg per day. Indeed, pharmacodynamic studies with the erlotinib support the selection of that dose (79). It is possible that the higher dose results in intratumor drug levels high enough to target the wild-type receptor. While the overall benefit of the agent in wild-type patients is small, it is still better than a placebo and enough to result in a statistically different outcome. In summary, while selecting the dose for a targeted agent based on toxicity criteria is indeed unsophisticated and crude, selecting a lower dose based on non-validated endpoints may be detrimental.

10.8.2 Rushing Too Fast to Phase III Trials was not Very Productive

After the conclusion of the IDEAL trials, with a ~10 percent response rate, the INTACT trials were launched. The only rationale for these studies was the notion that EGFR inhibition exerted synergistic or additive effects with chemotherapy in preclinical models. Once the gefitinib studies were ongoing, erlotinib followed the same approach. It is likely that the decisions to initiate these trials were more commercial strategies rather than scientific rationale. The synergistic effects in preclinical models were, for the most part, based on artificially EGFR dependent tumors and interpretation is further limited by issues of dose used. In retrospect, the Phase II studies of the combinations, completed after the Phase III studies, were not impressive and – it could be argued – indicated that mayor differences was not realistic. The lesson learned is that there should be more preliminary exploratory data before large studies that consume significant resources are launched. A strategy used more frequently (everyday) is the randomizec Phase II design which provides a less biased estimation of the activity of a combination, and that may decrease the risk of negative Phase II studies.

10.8.3 Predicting Which Patients are More Likely to Respond

At the time clinical trials with EGFR TKI were launched, there was very little information as to which were the markers predictive of outcome. This information was deciphered after treatment of many patients in multiple clinical trials. The first lesson learned is that it is possible to find these predictors and that genetic-based

markers are probably the more fruitful. The key question from a clinical development perspective is if trials should be conducted in selected patient populations or in general groups. One factor to consider is the rationale behind the target-drug interaction. In situations such as bcr/abl, c-kit or HER2 in which it was relatively clear that the agents would work in selected populations, the decision to base the development on such criteria was right (80, 81). For many other targeted agents, however, such knowledge is not available at the time studies commence or there are no well-validated tests to measure the target in tumor tissues. It is clear that more preclinical studies oriented to define biomarkers of response and not just activity is needed. In addition, in situations where a biomarker for patient selection is not available; every effort should be made to collect tumor tissue for translational studies. Indeed, modern clinical trials should obligatorily make an effort to collect such tissues so that when activity is observed, the cause can be explored.

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