

Molecularly targeted approaches herald a new era of non-small-cell lung cancer treatment

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Abstract: The discovery of activating mutations in the epidermal growth-factor receptor (*EGFR*) gene in 2004 opened a new era of personalized treatment for non-small-cell lung cancer (NSCLC). *EGFR* mutations are associated with a high sensitivity to *EGFR* tyrosine kinase inhibitors, such as gefitinib and erlotinib. Treatment with these agents in *EGFR*-mutant NSCLC patients results in dramatically high response rates and prolonged progression-free survival compared with conventional standard chemotherapy. Subsequently, echinoderm microtubule-associated protein-like 4 (*EML4*)–anaplastic lymphoma kinase (*ALK*), a novel driver oncogene, has been found in 2007. Crizotinib, the first clinically available *ALK* tyrosine kinase inhibitor, appeared more effective compared with standard chemotherapy in NSCLC patients harboring *EML4-ALK*. The identification of *EGFR* mutations and *ALK* rearrangement in NSCLC has further accelerated the shift to personalized treatment based on the appropriate patient selection according to detailed molecular genetic characterization. This review summarizes these genetic biomarker-based approaches to NSCLC, which allow the instigation of individualized therapy to provide the desired clinical outcome.

Keywords: non-small-cell lung cancer, epidermal growth factor receptor, *ALK* rearrangement, gefitinib, erlotinib, crizotinib

Introduction

Non-small-cell lung cancer (NSCLC) has a poor prognosis and remains the leading cause of death related to cancer worldwide.¹ For most individuals with advanced, metastatic NSCLC, cytotoxic chemotherapy is the mainstay of treatment on the basis of the associated moderate improvement in survival and quality of life.^{2,3} However, the outcome of chemotherapy in such patients has reached a plateau in terms of overall response rate (25%–35%) and overall survival (OS; 8–10 months).⁴ This poor outcome, even for patients with advanced NSCLC who respond to such chemotherapy, has motivated a search for new therapeutic approaches.

Recent years have seen rapid progress in the development of new treatment strategies for advanced NSCLC, in particular the introduction of molecularly targeted therapies and appropriate patient selection. First, the most important change has been customization of treatment according to patient selection based on the genetic profile of the tumor. Small-molecule tyrosine kinase inhibitors (TKIs) that target the epidermal growth-factor receptor (*EGFR*), such as gefitinib and erlotinib, are especially effective in the treatment of NSCLC patients who harbor activating *EGFR* mutations. In addition, TKIs that target the receptor tyrosine kinase anaplastic lymphoma kinase (*ALK*) have a high response rate and markedly prolong OS in NSCLC

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patients positive for *ALK* rearrangement. The identification of *EGFR* mutations and *ALK* rearrangement in individuals with NSCLC has thus accelerated the shift to personalized treatment for this condition. Second, recent studies have demonstrated the efficacy of monoclonal antibodies, such as bevacizumab, in combination with first-line platinum-based chemotherapy in advanced non-squamous NSCLC. Third, the introduction of pemetrexed has revealed differences in OS based on histological subtype of NSCLC, with the efficacy of pemetrexed being superior to that of gemcitabine in combination with cisplatin in individuals with non-squamous NSCLC (especially adenocarcinoma), and the opposite being true for those with squamous cell carcinoma. Together, these developments show that treatment for NSCLC is evolving toward a more personalized approach based on histological subtype or the molecular or genetic profile of the tumor. This review summarizes new treatment approaches to NSCLC, focusing on the development of molecularly targeted agents, including EGFR-TKIs and ALK-TKIs, both of which are key agents for personalized (genetic information-based) therapies in individuals with this condition.

EGFR-TKIs

In 2004, three groups in the US reported the landmark findings that a subset of NSCLC patients harbor activating mutations of *EGFR*,⁵⁻⁷ and those tumors positive for such mutations are highly sensitive to EGFR-TKIs, such as gefitinib and erlotinib. Indeed, most NSCLC patients who experienced a marked response to EGFR-TKIs were found to harbor *EGFR* mutations. *EGFR* mutations are present predominantly in women, never-smokers, individuals with adenocarcinoma, and those of East Asian ethnicity.⁸⁻¹¹ It has now been demonstrated definitively that the efficacy of EGFR-TKIs is largely dependent on the presence of an *EGFR* mutation in the tumor.

The role of EGFR-TKI treatment for NSCLC positive for *EGFR* mutations

Subsequent to the discovery of *EGFR* mutations in a subset of NSCLC patients in the relatively small studies published in 2004, several prospective single-arm studies showed significant efficacy of EGFR-TKIs, with a high response rate of 55%–91%, in such patients.^{8,12-18} Our group analyzed individual patient data from seven prospective phase II trials of gefitinib monotherapy in Japan, including a total 148 *EGFR* mutation-positive patients.¹⁹ The Iressa (gefitinib) Combined Analysis of Mutation Positives (I-CAMP) study showed that the overall response rate for gefitinib was 76.4%.

With a median follow-up of 20.7 months, the patients treated with gefitinib showed a highly favorable progression-free survival (PFS) of 9.7 months and OS of 24.3 months. Erlotinib yielded similar results, with a median survival time of more than 2 years, in a large prospective study of the Spanish Lung Cancer Group performed with 217 *EGFR* mutation-positive NSCLC patients.⁸ A pooled analysis of five additional trials also showed that *EGFR* mutations are a better indicator of clinical outcome in NSCLC patients than are such clinical predictors as sex, tumor histology, smoking status, and ethnicity.²⁰ These data suggested that there is no major ethnic difference in the pronounced clinical effects of EGFR-TKI treatment in *EGFR* mutation-positive patients, even though the *EGFR* mutation rate differs markedly between East Asian and Western countries, with a lower frequency in the latter.

Taken together, evidence thus supports a key role for EGFR-TKIs as a new and highly effective treatment option for NSCLC patients who harbor activating *EGFR* mutations. The clinical application of these findings, however, raises important issues with regard to molecular analysis of the tumor before initiation of treatment, drug selection, and treatment sequence.

The need for molecular analysis prior to treatment with EGFR-TKIs

Two pivotal phase III trials compared gefitinib with standard platinum chemotherapy in the first-line setting for individuals with advanced NSCLC.^{21,22} The patients enrolled in these trials were selected according to clinical characteristics associated with a high prevalence of activating *EGFR* mutations. The largest of these phase III trials, the Iressa pan-Asia Study (IPASS),²² assigned 1217 East Asian never-smokers (or former light smokers) with previously untreated lung adenocarcinoma to either gefitinib or carboplatin plus paclitaxel. First-line gefitinib treatment yielded a significantly higher overall response rate and longer PFS, the primary end point of the study, compared with chemotherapy. However, the PFS curves crossed at ~6 months after the start of treatment, favoring the chemotherapy group during the initial 6 months and gefitinib thereafter, indicating that the beneficial effect of gefitinib on PFS might be limited to those patients who harbored activating *EGFR* mutations. A total of 683 (56%) tumor samples were obtained from the patients enrolled in this study for exploratory biomarker analysis.²³ *EGFR* mutational status was evaluated in 437 patients, of whom 261 (60%) were found to harbor an activating mutation. In comparison with chemotherapy, gefitinib treatment improved PFS in

patients with *EGFR* mutations, whereas it was inferior to chemotherapy in those without such mutations (Table 1).²² Even in a patient population selected on the basis of a clinical characteristic associated with a favorable outcome of *EGFR*-TKI treatment, heterogeneity was clearly apparent between the patients with or without *EGFR* mutations. On the other hand, there was no difference in OS between the two treatment groups for *EGFR* mutation-positive patients in the overall analysis (Table 1).

The First-Line Single-Agent Iressa Versus Gemcitabine and Cisplatin Trial in Never-Smokers with Adenocarcinoma of the Lung (First-SIGNAL),²¹ a smaller Asian trial, obtained results similar to those of the IPASS trial. The eligibility criteria for this study were also similar to those of the IPASS trial. A total of 313 Korean never-smokers with adenocarcinoma were randomized to first-line treatment with either gefitinib or gemcitabine and cisplatin. Overall, OS and PFS did not differ significantly between the two groups. Among 96 patients (31%) whose tumors were analyzed for *EGFR* mutations, 42 individuals (44%) were positive for such mutations (Table 1). As in the IPASS study, gefitinib prolonged PFS in *EGFR* mutation-positive patients, although the difference between the two treatment arms was not statistically significant. However, PFS in the *EGFR* mutation-negative patients was worsened by gefitinib compared with chemotherapy. In this study, there was a higher response rate

to gefitinib in the mutation-negative population compared with that observed in the IPASS study, which together with the lack of a significant difference in PFS between gefitinib and chemotherapy in the mutation-positive population was due to a higher false-negative rate for *EGFR* mutations that resulted from non-centralized testing. The remarkable finding from both these trials, however, was that patient selection based on clinical characteristics alone was insufficient to predict accurately the benefit of *EGFR*-TKI treatment,²⁴ indicating that molecular analysis of *EGFR* mutational status is mandatory prior to treatment.

EGFR-TKIs in the first-line setting for *EGFR* mutation-positive patients with advanced NSCLC

The results of the IPASS study indicated that *EGFR*-TKIs have promising efficacy for individualized treatment of advanced NSCLC positive for *EGFR* mutations. We conducted a randomized phase III trial (WJTOG3405) that compared gefitinib with platinum-based chemotherapy (cisplatin plus docetaxel) in the first-line setting for 172 patients with advanced NSCLC positive for *EGFR* mutations.²⁵ This study met the primary end point in that PFS was found to be significantly longer in the gefitinib group than in the chemotherapy group (hazard ratio 0.489, 95% confidence interval 0.37–0.71; $P < 0.0001$). It did not,

Table 1 Results of phase III trials comparing epidermal growth-factor receptor (EGFR)-tyrosine kinase inhibitors with chemotherapy as first-line treatment in non-small-cell lung cancer patients with *EGFR* mutations

Authors	Trial	Regimens	Patients	Method to detect <i>EGFR</i> mutation	ORR (%)	PFS		OS	
						Months	HR (95% CI)	Months	HR (95% CI)
Mok et al, ²²	IPASS	Gefitinib	132	SARMS	71.2	9.5	0.48	21.6	1.00
Fukuoka et al ²³		CBDCA plus PAC	129		47.3	6.3	(0.34–0.67) $P < 0.001$	21.9	(0.76–1.33) $P = 0.990$
Han et al ²¹	First-SIGNAL	Gefitinib	26	Direct sequencing	84.6	8.0	0.54	27.2	1.043
		CDDP plus GEM	16		37.5	6.3	(0.269–1.100) $P = 0.086$	25.6	(0.498–2.182) –
Mitsudomi et al ^{25,26}	WJTOG3405	Gefitinib	86	Various methods	62.1	9.6	0.520	38.8	1.185
		CDDP plus DOC	86		32.1	6.6	(0.378–0.715) $P < 0.001$	35.5	(0.767–1.829) $P = 0.443$
Maemondo et al ²⁷	NEJ002	Gefitinib	114	PNA-LNA PCR clamp	73.7	10.8	0.322	27.7	0.887
		CBDCA plus PAC	114		30.7	5.4	(0.236–0.438) $P < 0.001$	26.6	(0.634–1.241) $P = 0.483$
Zhou et al ³⁰	OPTIMAL	Erlotinib	82	Direct sequencing	83.0	13.1	0.16	22.7	1.04
		CBDCA plus GEM	72		36.0	4.6	(0.10–0.26) $P < 0.0001$	28.8	(0.69–1.58) $P = 0.691$
Rosell et al ³²	EUROTAC	Erlotinib	86	Various methods	64.0	9.7	0.37	19.3	1.04
		CDDP-based	87		15.0	5.2	(0.25–0.54) $P < 0.0001$	19.5	(0.65–1.68) $P = 0.870$

Abbreviations: CBDCA, carboplatin; PAC, paclitaxel; CDDP, cisplatin; GEM, gemcitabine; DOC, docetaxel; ORR, objective response rate; PFS, progression-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; SARMS, Scorpion amplification-refractory mutation system; PNA-LNA PCR, peptide nucleic acid-locked nucleic acid polymerase chain reaction.

however, reveal a statistically significant improvement in OS in the gefitinib group. Updated OS analysis of the study showed that the median OS of patients who received gefitinib was 35.5 months, which did not differ significantly from the value of 38.8 months for those who received standard chemotherapy (hazard ratio 1.185, 95% confidence interval 0.767–1.829; $P = 0.443$)²⁶ (Table 1). Similar findings on the superiority of EGFR-TKIs were obtained in another Japanese phase III study (NEJ002),²⁷ in which 228 NSCLC patients with *EGFR* mutations were assigned to gefitinib or to carboplatin and paclitaxel. The recent updated analysis for this study again demonstrated no significant difference in OS between the two groups. Whereas the median PFS for the gefitinib group was 10.8 months compared with 5.4 months for the chemotherapy group (hazard ratio 0.322, 95% confidence interval 0.236–0.438; $P < 0.001$), the median OS for gefitinib and for chemotherapy was 27.7 and 26.6 months, respectively.²⁸ When survival curves are virtually identical for different groups in a randomized trial for advanced NSCLC, an improvement in quality of life or cancer-related symptoms becomes an important issue. Whereas the WJTOG3405 trial did not provide data on quality of life, the NEJ002 study found that quality of life was maintained for a longer time in patients receiving gefitinib than in those receiving standard systemic chemotherapy.²⁹ Several other phase III trials have also shown that EGFR-TKIs improve quality of life compared with chemotherapy.^{21,22,30,31} Superiority of erlotinib over platinum-based chemotherapy in terms of overall response rate and PFS but not OS has also been demonstrated in phase III trials^{30,32} (Table 1).

None of the phase III trials that have compared EGFR-TKIs with standard platinum-based chemotherapy in patients with *EGFR* mutations has revealed a benefit in terms of OS. Failure to translate an extended PFS into an obvious survival benefit in these studies is accounted for by a high frequency of crossover treatment with EGFR-TKIs after disease progression in the chemotherapy group. However, this finding does not mean that EGFR-TKIs have little value in the first-line setting. In clinical practice, not all patients with *EGFR* mutations who receive standard chemotherapy in the first-line setting will be suitable for subsequent treatment with EGFR-TKIs, as a result of a rapid deterioration of their general condition and performance status due to disease progression. The chance to administer EGFR-TKIs in such patients would thus be missed. Subset analysis of the NEJ002 trial recently showed that the impact of platinum-based chemotherapy on OS was not greater than that of gefitinib in NSCLC patients with *EGFR* mutations.²⁸ The median OS of

patients who were treated with gefitinib in any line but who did not receive platinum-based chemotherapy was more than 2 years, which is an improvement compared with historical data obtained when EGFR-TKIs were not available.³³ EGFR-TKIs are thus now globally recognized as important drugs and the standard first-line treatment for advanced NSCLC patients with *EGFR* mutations.

Resistance to EGFR-TKIs

Most NSCLC patients who harbor activating *EGFR* mutations, including deletions in exon 19 or the point mutation L858R in exon 21, experience an initial marked response to the EGFR-TKIs gefitinib or erlotinib. However, almost all such individuals eventually develop acquired resistance to these drugs within 1 year. In addition, 20%–30% of NSCLC patients with *EGFR* mutations do not show an initial response to EGFR-TKIs.^{22,25,27} Therapeutic strategies to overcome EGFR-TKI resistance in NSCLC patients with *EGFR* mutations have been developed on the basis of the biological mechanisms of such resistance, which include a T790M secondary mutation in *EGFR* as well as amplification of the gene for the receptor tyrosine kinase MET, which serves as the receptor for hepatocyte growth factor.

T790M secondary *EGFR* mutation as a mechanism of EGFR-TKI resistance

A secondary point mutation of *EGFR* that results in the substitution of methionine for threonine at amino acid position 790 (T790M) was the first identified mechanism of acquired EGFR-TKI resistance in NSCLC patients.^{34,35} About 50%–70% of NSCLC patients who develop acquired resistance to EGFR-TKIs have been found to harbor the T790M secondary mutation, with the mutation not being present in tumor specimens obtained before EGFR-TKI treatment.^{36,37} The T790M mutation has also been detected, however, in a small proportion of NSCLC patients who have not yet received any treatment.³⁸ Several highly sensitive methods, compared with direct sequencing, have recently been developed to detect a low frequency of T790M in genetically heterogeneous clinical specimens. These methods include polymerase chain reaction (PCR) invader,³⁹ peptide nucleic acid-locked nucleic acid PCR clamp,⁴⁰ and Cycleave PCR assays.³⁷ The Scorpion amplification-refractory mutation system assay identified T790M in circulating tumor cells of NSCLC patients before EGFR-TKI treatment.⁴¹ Matrix-assisted laser desorption ionization–time of flight mass spectrometry detected T790M in 25.2% of TKI-naïve NSCLC patients who harbored activating *EGFR* mutations.⁴²

The presence of the T790M mutation in NSCLC patients before treatment was found to be associated with a significantly shorter PFS after initiation of EGFR-TKI treatment.^{41,42} These observations thus suggest that T790M contributes not only to acquired resistance to EGFR-TKIs but also to intrinsic resistance to these drugs.

Similar to mutations in *BCR-ABL* (T315I)⁴³ or in *KIT* (T670I)⁴⁴ that underlie resistance to imatinib, T790M is thought to interfere with the binding of EGFR-TKIs at the adenosine triphosphate-binding cleft of EGFR.^{34,35,45} On the other hand, the affinity of this cleft for adenosine triphosphate is increased by T790M.⁴⁶ Treatment with irreversible EGFR-TKIs such as afatinib (BIBW2992)⁴⁷ and dacomitinib (PF00299804)^{48,49} is thought to be a potential approach to overcome the resistance conferred by T790M (Figure 1A). Although recent preclinical studies have demonstrated only limited activity of irreversible EGFR-TKIs alone in NSCLC cells positive for T790M,^{50,51} such studies have shown that combinations of these drugs with other agents – such as afatinib combined with cetuximab (a monoclonal antibody to EGFR)⁵² or with PI-103 (an inhibitor of signaling by phosphoinositide 3-kinase and mammalian target of

rapamycin)⁵⁰ – are more promising as a treatment option to overcome resistance conferred by T790M. In addition, treatment with heat-shock protein 90 inhibitors such as 17-DMAG is also thought to be a potential approach to counter the effect of T790M.⁵³ Furthermore, WZ4002, which selectively inhibits the activity of EGFR harboring activating mutations and T790M, has been identified as a candidate for translation to the clinic.⁵⁴

MET amplification and other mechanisms of EGFR-TKI resistance

MET amplification was identified as a mechanism of gefitinib resistance in 22% of NSCLC patients with acquired resistance to this drug.⁵⁵ Both *MET* and EGFR signaling were found to activate phosphoinositide 3-kinase via ErbB3 (also known as HER3) in gefitinib-resistant NSCLC cells positive for *MET* amplification.⁵⁵ The combination of gefitinib and the *MET* inhibitor PHA665752 was thus required to block survival signaling in these cells (Figure 1B).⁵⁵ In the clinical setting, a phase I/II trial of the *MET*-TKI crizotinib in combination with erlotinib is ongoing in patients with NSCLC (NCT00965731). The results of the phase I portion of a

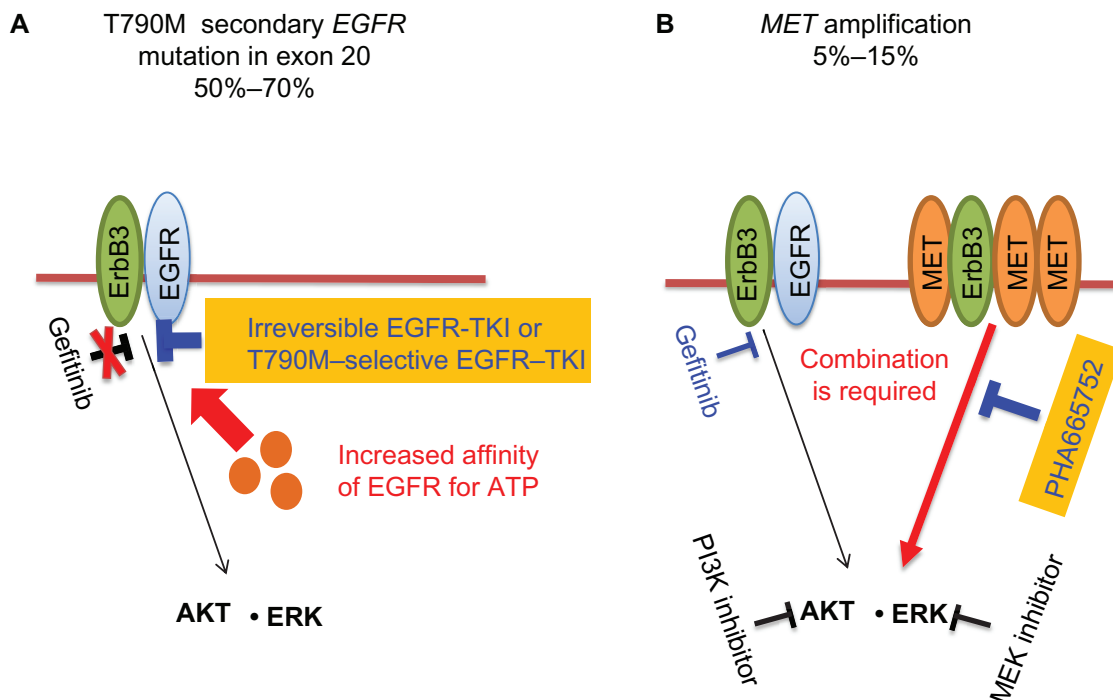


Figure 1 (A and B) Strategies to overcome acquired epidermal growth-factor receptor (EGFR)-tyrosine kinase inhibitor (TKI) resistance in non-small-cell lung cancer (NSCLC). (A) The T790M secondary mutation in exon 20 of EGFR is present in 50%–70% of NSCLC patients who acquire resistance to EGFR-TKIs, such as gefitinib or erlotinib. In such patients, gefitinib is not able to compete with adenosine triphosphate (ATP) for binding to the ATP-binding cleft of EGFR because of an increased affinity of this site for ATP. Treatment with irreversible EGFR-TKIs or EGFR-TKIs selective for EGFR harboring T790M is thus thought to represent a potential approach to overcome the resistance conferred by this mutation. (B) Amplification of *MET* is apparent in 5%–15% of NSCLC patients who acquire EGFR-TKI resistance. In this situation, *MET* signaling through ErbB3 (HER3) is activated in addition to EGFR signaling, with the result that both gefitinib and a *MET* inhibitor (such as PHA665752) are necessary to overcome the resistance conferred by *MET* amplification. The combination of inhibitors that block molecules that function downstream of both EGFR and *MET*, such as a phosphoinositide 3-kinase (PI3K) inhibitor combined with an MEK (ERK kinase) inhibitor, might also be an alternative approach to overcome the resistance induced by *MET* amplification.

phase I/II dose-finding study for crizotinib were reported at the 2012 Annual Meeting of the European Society for Medical Oncology.⁵⁶ This study allowed recruitment of NSCLC patients who had received prior EGFR-TKI therapy. Of the 25 enrolled patients, two showed a partial response and eight had stable disease. Although few studies have addressed other therapies to overcome resistance conferred by *MET* amplification, dasatinib, an inhibitor of the non-receptor tyrosine kinase Src, effectively inhibited the growth of gefitinib-resistant NSCLC cells positive for *EGFR* mutation and *MET* amplification, with this approach being based on the observation that Src acts downstream of both EGFR and MET in these cells.⁵⁷ Another preclinical study suggested that combination of gefitinib with the oral fluoropyrimidine derivative S-1 is a potential therapy to overcome acquired resistance due to *MET* amplification.⁵⁸

Several other possible mechanisms of acquired resistance to EGFR-TKIs – including upregulation of insulin-like growth-factor 1 receptor signaling,^{59–61} loss of the phosphatase PTEN,^{62,63} small-cell transformation,^{64–66} and overexpression of hepatocyte growth factor⁶⁷ – have been described. However, the mechanism of acquired EGFR-TKI resistance in ~30% of NSCLC patients remains unclear. Recent studies have implicated epithelial–mesenchymal transition (EMT) as a possible mechanism of acquired EGFR-TKI resistance in NSCLC cell lines.^{68,69} Furthermore, tumor cells having undergone EMT were detected in a subset of NSCLC patients who developed EGFR-TKI resistance.^{70,71} Although histone deacetylase inhibitors may help overcome EMT-related resistance to EGFR-TKIs in NSCLC,⁶⁹ further studies are required to provide a better understanding of the role of EMT in such resistance and to identify novel therapeutic strategies to overcome it.

ALK inhibitors

Role of the *EML4*-*ALK* fusion gene in NSCLC

The *ALK* gene undergoes transforming rearrangements that generate fusion genes in several human hematologic and solid malignancies.^{72,73} The partner genes in such fusions with *ALK* include *NPM*, *TPM3*, and *CLTC*. In 2007, Soda et al⁷⁴ identified a new *ALK* rearrangement that results from an inversion within chromosome 2p and generates a transforming fusion of *ALK* with the echinoderm microtubule-associated protein-like 4 gene (*EML4*) in NSCLC. Several in-frame fusion variants of *EML4*-*ALK* have been found to be generated as a result of diversity in the breakpoint–fusion point of *EML4*, and other rare non-*EML4* fusion partners of

ALK, including *KIF5B* and *TFG*, have since been identified in NSCLC.^{75–77}

The reported incidence of *EML4*-*ALK* has varied among studies as a result of different procedures adopted for detection of the fusion gene.⁷⁸ The prevalence of *ALK* rearrangement as detected either by reverse transcription and PCR analysis or by fluorescence in situ hybridization (FISH) is relatively low (in the order of 3%–5%), however, in unselected patients with NSCLC.⁷⁸ *ALK* status as determined by FISH, which is regarded as the global standard for detection of *ALK* rearrangement, is currently considered the key predictive marker for treatment of NSCLC patients with an ALK-TKI.⁷⁹ Indeed, positive identification of *EML4*-*ALK* by FISH was associated with a high sensitivity to a small-molecule inhibitor of ALK tyrosine kinase activity in a clinical trial.⁷⁹ Although *EML4*-*ALK* is present in only a small proportion of unselected NSCLC patients, the clinical characteristics of NSCLC patients harboring *EML4*-*ALK* are highly similar to those of such patients who harbor activating *EGFR* mutations.^{76,79,80} Both types of gene alteration are thus found most frequently in patients with adenocarcinoma and in those who are never- or light smokers. Individuals with *EML4*-*ALK* also tend to be younger than unselected NSCLC patients. With rare exceptions, the presence of *EML4*-*ALK* appears to be mutually exclusive with that of *EGFR* or *KRAS* mutations.⁸¹ Furthermore, whereas *EGFR* mutations are present more frequently in East Asian populations than in Caucasians, no ethnic differences in the frequency of *EML4*-*ALK* among NSCLC patients have been reported. With regard to its associated pathological features, *EML4*-*ALK* tends to be found in lung adenocarcinoma with a mucinous cribriform pattern and signet-ring cells,^{82–84} although it has also been detected in other pathological subtypes of NSCLC and other types of cancer, including breast and colorectal tumors.^{85,86}

Crizotinib

Whereas several ALK inhibitors have already been introduced into clinical trials, crizotinib was the first ALK-TKI to be so evaluated. Crizotinib was initially designed as an inhibitor of MET and is thus also known as a dual inhibitor of both ALK and MET kinases.^{87,88} A phase I trial revealed marked therapeutic efficacy of crizotinib in patients with NSCLC positive for *EML4*-*ALK* (Table 2), with an overall response rate of 60.8% and a disease control rate of up to 90%.^{79,89} Crizotinib was thus approved as a therapeutic drug for certain patients with NSCLC positive for *EML4*-*ALK* by the US Food and Drug Administration.

Table 2 Clinical trials of crizotinib treatment for advanced non-small-cell lung cancer positive for anaplastic lymphoma kinase rearrangement

Phase	Regimens	Patients	ORR (%)	PFS (months)	OS (months)	References
I	Crizotinib	149	60.8	9.7	Not achieved	79,89
II	Crizotinib	261	59.8	8.1	Not achieved	91
III	Crizotinib	172	65.7	7.7	20.3	90
	Docetaxel	72	6.9	2.6	22.8	
	Pemetrexed	99	29.3	4.2		

Abbreviations: PFS, progression-free survival; OS, overall survival; ORR, objective response rate.

These remarkable findings of crizotinib activity led to two subsequent phase III clinical trials of this drug. The Profile 1007 trial, a global, randomized phase III study with a primary end point of PFS, was designed to compare crizotinib with pemetrexed or docetaxel chemotherapy in patients with advanced NSCLC positive for *EML4-ALK* in the second-line setting.⁹⁰ Patients treated with docetaxel or pemetrexed who developed progressive disease were allowed to receive crizotinib in a companion phase II trial (Profile 1005) that was designed to enroll *EML4-ALK*-positive NSCLC patients who were not eligible for Profile 1007.⁹¹ The purpose of the phase II study was to evaluate crizotinib in terms of efficacy and safety through analysis of adverse events. The second phase III trial, Profile 1014, is ongoing and compares crizotinib with cisplatin/carboplatin and pemetrexed as a first-line treatment for patients with advanced NSCLC harboring *EML4-ALK* as detected by FISH (NCT01154140). The primary end point of this trial is also PFS, as was the case for EGFR-TKI trials, because of a high potential for crossover treatment. The Profile 1007 trial showed that crizotinib significantly prolonged PFS and had a higher overall response rate in comparison with standard single-agent chemotherapy (Table 2). However, no statistically significant difference in OS was observed between crizotinib and chemotherapy as a result of the anticipated high level of crossover, although the interim analysis of OS was premature. Furthermore, the improvement in both lung cancer-related symptoms and quality of life relative to baseline observed with crizotinib was significantly greater than that achieved with chemotherapy. These findings thus demonstrated an efficacy for crizotinib in patients with *ALK* rearrangement-positive NSCLC similar to that for EGFR-TKIs in those with *EGFR* mutation-positive NSCLC. These thus established crizotinib treatment as the standard of care for previously treated patients with advanced NSCLC harboring *EML4-ALK*.

Other ALK inhibitors

As of March 2013, at least five additional distinct inhibitors of ALK were undergoing evaluation in early clinical trials

worldwide (Table 3). CH5424802 is a potent, selective inhibitor of ALK that was found to show preferential antitumor activity for *ALK* rearrangement-positive cancer cells, such as *EML4-ALK*-positive NSCLC cells and *NPM-ALK*-positive anaplastic large-cell lymphoma cells, in a preclinical study.⁹² This agent also potently inhibits the activity of ALK containing the L1196M “gatekeeper mutation,” which confers clinical resistance to ALK inhibitors. A phase I trial revealed that CH5424802 had marked efficacy and was well tolerated in patients with *ALK* rearrangement-positive NSCLC.⁹³ Preliminary results of a phase II trial have also been presented.⁹⁴ As of March 2012, 34 patients with *ALK* rearrangement-positive NSCLC previously untreated with ALK inhibitors had been enrolled and received CH5424802 at 300 mg twice daily until the development of progressive disease or unacceptable toxicity. Most of the patients were never-smokers with good performance status and had previously received extensive chemotherapy. Of the first 15 patients receiving this agent, one individual achieved a complete response and ten individuals showed a partial response, yielding a response rate of 73.3%. Most treatment-related adverse events were of grade 1. No dose reductions were necessary, although two cases of grade 3 neutropenia occurred. With regard to eye disorders, which are frequently observed with crizotinib, only one case of grade 1 was described.

LDK378 is another potent and selective small-molecule ALK inhibitor (median inhibitory concentration of 0.00015 μ M) that does not inhibit MET. A phase I trial has

Table 3 Anaplastic lymphoma kinase (ALK) inhibitors in early clinical development for treatment of *ALK* rearrangement-positive non-small-cell lung cancer

Inhibitor	Phase	Company	References
CH5424802	II	Chugai	93,94
LDK378	I	Novartis	95
ASP3026	I	Astellas Pharma	100
AP26113	I/II	Ariad	98
X396	I	Xcovery	99

been initiated to determine the maximum tolerated dose and safety profile of this agent in patients with solid tumors positive for genetic alterations of *ALK*, including gene rearrangement.⁹⁵ Patients previously untreated with *ALK* inhibitors or those with disease relapse after previous treatment with such an inhibitor are eligible for enrollment in the trial. As of August 2012, 79 *ALK* rearrangement-positive NSCLC patients, including 56 who had received prior crizotinib treatment, were enrolled and had been administered LDK378 in a dose range of 50–750 mg/day. At doses of ≥ 400 mg, steady-state concentrations of the drug in plasma exceeded efficacious levels determined in vivo. Among 59 evaluable NSCLC patients, there were 24 responses, yielding a response rate of 40.7%. Among 45 patients with *ALK* rearrangement-positive NSCLC who had experienced progression following crizotinib treatment and received LDK378 at ≥ 400 mg/day, preliminary responses were apparent in 21 patients (46.7%). Dose-limiting toxicity occurred at doses of ≥ 400 mg/day and included diarrhea, nausea, vomiting, dehydration, and elevation of alanine aminotransferase levels. The maximum tolerated dose was 750 mg/day. These preliminary results suggest that LDK378 may prove effective for the treatment of patients with *ALK* rearrangement-positive NSCLC who develop acquired resistance to crizotinib. Two phase II trials of LDK378 at a dose of 750 mg/day are ongoing for *ALK* rearrangement-positive NSCLC patients previously treated either with crizotinib or with standard chemotherapy.

AP26113 is a synthetic and highly selective small-molecule inhibitor designed to target *ALK*. It is actually a dual inhibitor for both *ALK* and *EGFR* with activating mutations, whereas it does not inhibit wild-type *EGFR*. Furthermore, it potently inhibits TKI-resistant forms of these kinases harboring gatekeeper mutations (L1196M in *ALK* and T790M in *EGFR*).^{96,97} As of September 2012, 15 patients with advanced malignancies, including eleven individuals with NSCLC, had been enrolled in a phase I/II trial of AP26113.⁹⁸ The NSCLC patients included four with *ALK* rearrangement-positive tumors that developed resistance to prior crizotinib treatment. All patients received AP26113 at doses of 30–120 mg/day. All four *ALK* rearrangement-positive NSCLC patients showed partial responses, with one patient treated at a dose of 60 mg and the other three at 90 mg. The efficacy and safety of this agent were evaluated at a dose of 120 mg. Neither dose-limiting toxicities nor treatment-related serious adverse events were observed, with the most common adverse events being fatigue and nausea. The results of the phase I portion of the trial thus indicate that AP26113 is effective with acceptable toxicity,

and they suggest it may have potential for the treatment of patients with *ALK* rearrangement-positive NSCLC who have experienced disease progression while receiving crizotinib. The phase II portion will evaluate the efficacy and safety of AP26113 in several cohorts.

Phase I clinical trials of the remaining two *ALK*-specific agents – X-396⁹⁹ and ASP3026¹⁰⁰ – have been initiated in patients with *ALK* rearrangement-positive advanced solid tumors and NSCLC, respectively.

Conclusion

Over the course of the last several years, the introduction of molecularly targeted agents such as gefitinib, erlotinib, and crizotinib has resulted in marked changes in treatment approaches to NSCLC. The introduction of these agents into the clinic has followed the identification of genetic changes that give rise to NSCLC and has been accompanied by appropriate patient selection. Such drugs are expensive, however, and their use is limited to subsets of patients in whom the target has undergone activating changes and become an “essential growth driver” for the cancer. Personalized therapy is based on the notion that appropriate patient selection according to detailed molecular genetic characterization will allow the instigation of individualized therapy to provide the desired clinical outcome. Treatment of NSCLC will thus come to rely more and more on a genetic biomarker-based approach to prolong survival. The advent of next-generation technologies for genetic characterization of each patient will be important to facilitate further development of individualized treatment of NSCLC with molecularly targeted agents.

Disclosure

The authors report no conflicts of interest in this work.

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