The potential for crizotinib in non-small cell lung cancer: a perspective review

Yung-Jue Bang

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Abstract: Tyrosine kinases have a crucial role as key regulators of signaling pathways that influence cell differentiation and growth. Dysregulation of tyrosine kinase-mediated signaling is understood to be an important oncogenic driver. Genetic rearrangements involving the tyrosine kinase *anaplastic lymphoma kinase* (*ALK*) gene occur in non-small cell lung cancer (NSCLC), anaplastic large cell lymphomoas, inflammatory myofibroblastic tumors, and other cancers. Cells with abnormal ALK signaling are sensitive to ALK inhibitors such as crizotinib. This review will highlight the discovery of the fusion between echinoderm microtubule-associated protein-like 4 (EML4) and ALK as an oncogenic driver, recognition of other *ALK* gene rearrangements in NSCLC, and the confirmation that crizotinib is an effective treatment for patients with *ALK*-positive NSCLC. Work is underway to further define the role for crizotinib in the treatment of *ALK*-positive lung cancer and other cancers and to investigate the molecular mechanisms for resistance to ALK inhibition with crizotinib.

Keywords: anaplastic lymphoma kinase, carcinoma, crizotinib, non-small cell lung cancer, tyrosine kinase inhibitor

Introduction

Lung cancer has long been the most common cancer and cause of cancer-related deaths worldwide, with 1.61 million new cases and 1.38 million deaths in 2008 alone, representing 12.7% of new cancers and 18.2% of cancer mortality [Ferlay *et al.* 2010]. Approximately 85% of lung cancers are non-small cell lung cancer (NSCLC) and the majority of patients are diagnosed at an advanced stage [Subramanian and Govindan, 2008; Govindan *et al.* 2006; Yang *et al.* 2005]; 5-year survival for patients in the USA with NSCLC is approximately 16% [American Cancer Society, 2011]. Current treatments for NSCLC may extend survival but are rarely curative [Subramanian and Govindan, 2008; Greenlee *et al.* 2000].

NSCLC includes the adenocarcinoma, squamous-cell, and large-cell histological subtypes and, historically, these factors plus performance status have largely determined the choice of cytotoxic chemotherapy regimens for patients. NSCLC cytotoxic chemotherapy is currently focused on the use of platinum doublet chemotherapy, with or without bevacizumab [Langer *et al.* 2010].

Where NSCLC was previously considered to be a single disease treated with standard cytotoxic chemotherapy, it is now becoming more appropriate to consider NSCLC as a collection of disease subtypes according to the driving oncogenic aberration, and to select treatment accordingly. Crucially, the Iressa Pan Asia Study (IPASS), which compared the efficacy of the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) gefitinib with carboplatin plus paclitaxel in pulmonary adenocarcinoma, demonstrated not only that gefitinib was significantly more effective than chemotherapy first line in patients with *EGFR* mutations [hazard ratio (HR) for progression or death 0.75; $p < 0.001$] but also that, in patients without *EGFR* mutations, chemotherapy was superior to gefitinib [HR 2.85; 95% confidence interval (CI) 2.05 to 3.98; $p < 0.001$] [Mok *et al.* 2009]. Two Japanese studies have shown gefitinib to be more effective than platinum/taxane doublet chemotherapy as first-line treatment for patients with NSCLC harboring *EGFR* mutations; significant improvements in progression-free survival (PFS) were observed for gefitinib compared with both carboplatin/paclitaxel (PFS 10.8 *versus* 5.4 months; HR: 0.3; 95% CI 0.22 to 0.41; $p < 0.001$)

Correspondence to:
Yung-Jue Bang, MD
Department of Internal Medicine, Seoul National University College of Medicine, 101 Daehak-ro, Jongno-gu, Seoul 110-744, Korea
bangyj@snu.ac.kr

[Maemondo *et al.* 2010] and cisplatin/docetaxel (PFS 9.2 *versus* 6.3 months; HR: 0.49; 95% CI 0.34 to 0.71; $p < 0.001$) [Mitsudomi *et al.* 2010]. The CALGB 30406 study is investigating the efficacy of the EGFR TKI erlotinib administered alone and in combination with carboplatin/paclitaxel in never/former light smokers with chemotherapy-naïve, advanced NSCLC, and has reported a significantly increased PFS for patients with *EGFR* mutations compared with wild-type *EGFR* [Janne *et al.* 2010]. Interestingly, in this study erlotinib monotherapy had similar efficacy to combination therapy in *EGFR*-mutated tumors. The OPTIMAL study has compared erlotinib with gemcitabine/carboplatin in Chinese patients with untreated *EGFR*-mutated NSCLC, finding a considerable PFS advantage for erlotinib compared with chemotherapy [median PFS 13.1 months (erlotinib) *versus* 4.6 months (gemcitabine/carboplatin); HR: 0.16; 95% CI 0.10 to 0.26; $p < 0.0001$; $N = 154$] [Zhou *et al.* 2010]. Thus, the selection of chemotherapy regimens according to tumor histology does not necessarily represent the best choice for patients, and the selection of a better treatment option requires screening to accurately identify the disease driver.

Despite the growth in understanding of tyrosine kinases, their role in tumor development, and the increasing success of tyrosine kinase-based therapeutic agents, the precise kinases that drive most solid tumors remain unclear. This limits the identification of drug targets and prediction of response. Research carried out over the last 15 years, however, has shed much light on the expression of these proteins in cancer. The nucleophosmin (NPM)–anaplastic lymphoma kinase (ALK) fusion protein was first identified as a neoplastic agent in patients with anaplastic large cell lymphoma (ALCL) [Shiota and Mori, 1996; Shiota *et al.* 1995; Morris *et al.* 1994], with the NPM–ALK protein resulting from the translocation t(2,5)(p23:q35), between the *ALK* gene on chromosome 2 and the *NPM* gene on chromosome 5 [Lamant *et al.* 1996; Shiota and Mori, 1996]. *ALK* has since been linked with many different fusion partners in different tumor types, including *TRK-fused gene* (*TFG*), *tropomyosin* (*TPM*)*3, TPM4, 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase* (*ATIC*), and *moesin* (*MSN*) in ALCL; *TPM3, TPM4, cysteinyl tRNA synthetase* (*CARS*), *Ran-binding protein 2* (*RANBP2*), and *clathrin heavy chain gene* (*CLTC*) in inflammatory myofibroblastic tumor; a variety of *ALK*-amplifying point mutations in neuroblastoma; *TPM4* in esophageal squamous tumor; *SQSTM1–ALK* fusion, *CLTC*, and *NPM* in diffuse large B-cell lymphoma; and *echinoderm microtubule-associated protein-like 4* (*EML4*) and *kinesin family member 5B* (*KIF5B*) in NSCLC [Takeuchi *et al.* 2011, 2009; Palmer *et al.* 2009; Webb *et al.* 2009; Mossé *et al.* 2008; Coffin *et al.* 2007; Rikova *et al.* 2007; Soda *et al.* 2007; Jazii *et al.* 2006; Li *et al.* 2004; Ma *et al.* 2003; Onciu *et al.* 2003; Gascoyne *et al.* 2003; Drexler *et al.* 2000]. A further solid tumor *ALK* fusion (*VCL–ALK* in renal cell carcinoma) has also recently been reported [Debelenko *et al.* 2011]. In NSCLC, specifically, a large-scale survey of tyrosine kinase signaling across 41 cell lines and over 150 tumors identified known oncogenic kinases such as EGFR and c-MET, together with novel ALK and ROS fusion proteins [Rikova *et al.* 2007].

The *EML4–ALK* fusion gene was identified as tumorigenic in NSCLC in 2007 [Soda *et al.* 2007; Rikova *et al.* 2007] and the development of a clinic-ready inhibitor targeting it, crizotinib (PF-02341066), has been rapid, with the first clinical trial data for crizotinib in patients who are *ALK* fusion gene-positive published in 2010 [Kwak *et al.* 2010]. Crizotinib is also a highly specific inhibitor of the receptor tyrosine kinase c-MET (hepatocyte growth factor receptor), and was previously studied as a c-*MET* inhibitor [Rodig and Shapiro, 2010]. Here I discuss *ALK* in NSCLC and look at the potential which crizotinib has to make a difference in the treatment of NSCLC, and the issues surrounding the emergence of this ALK inhibitor for the treatment of NSCLC in the future.

EML4–ALK in NSCLC and mode of action of crizotinib

EML4–ALK is an inversion, where the *EML4* gene is disrupted at intron 13 and is linked to intron 19 of the *ALK* gene [Ensembl Genome Browser, 2011] (previously identified as 'upstream of exon 20'), producing a gene of 3926 base pairs coding for a protein of 1059 amino acids [Soda *et al.* 2007].

Incorporation of *EML4–ALK* into mouse fibroblasts resulted in tumor formation when these cells were injected into mice. Subsequently, a number of variants of the fusion gene were identified, differing in the point at which the *EML4*

gene was disrupted [Soda *et al.* 2007]. Transgenic mice expressing alveolar epithelial *EML4–ALK* developed hundreds of adenocarcinoma nodules in both lungs within weeks of birth, and subsequent treatment with an ALK inhibitor rapidly reduced tumor size [Soda *et al.* 2008]. Similarly, injection of fibroblasts expressing *EML4–ALK* into nude mice led to tumor development and fatal respiratory failure, whereas treatment with the ALK inhibitor resulted in a reduction in tumor burden and prolonged survival [Soda *et al.* 2008].

The precise intracellular signaling pathways by which the EML4–ALK fusion protein induces tumor growth and development have not been fully characterized. Likewise, the physiological function of ALK is currently unknown but there is evidence that the *ALK* gene plays a role in the development of the nervous system [Pulford *et al.* 2004] and, in humans, ALK protein expression is confined to scattered cells within the central nervous system [Pulford *et al.* 1997]. Studies of *NPM–ALK* in ALCL have implicated RAS–mitogen-activated protein kinase, phospholipase C gamma–Src homology 2, phosphoinositide 3-kinase–AKT, signal transducer and activator of transcription 3, and nuclear interacting partner of ALK [Pulford *et al.* 2004; Ouyang *et al.* 2003]. It has recently been reported that interactions between NPM–ALK and growth factor receptor-bound protein 2 play a key role in the regulation of ALCL cell signaling and growth [Riera *et al.* 2010]. There is therefore abundant potential for ALK fusion proteins to generate tumor growth and proliferation.

Crizotinib is an orally active small-molecule inhibitor of ALK and the c-Met receptor tyrosine kinase [Christensen *et al.* 2007; Zou *et al.* 2007], and belongs to the 3-benzyloxy-2-aminopyridine series of kinase inhibitors [Timofeevski *et al.* 2009]. Crizotinib is highly selective for ALK and c-Met kinases, with 50% inhibitory concentration values for ALK and c-Met of 5–20 nM, compared with values at least 20-fold higher for other kinases [Christensen *et al.* 2007; Zou *et al.* 2007]. Crizotinib acts by binding to the adenosine triphosphate (ATP) binding site (the 'ATP binding pocket') of the ALK enzyme (Figure 1), thereby preventing binding of ATP and subsequent autophosphorylation, which is required for activation of the enzyme. Crizotinib and the ALK inhibitor, TAE684, have been shown to inhibit tumor growth in the H2228 and H3122 NSCLC tumor models [Li *et al.* 2011; McDermott *et al.* 2008].

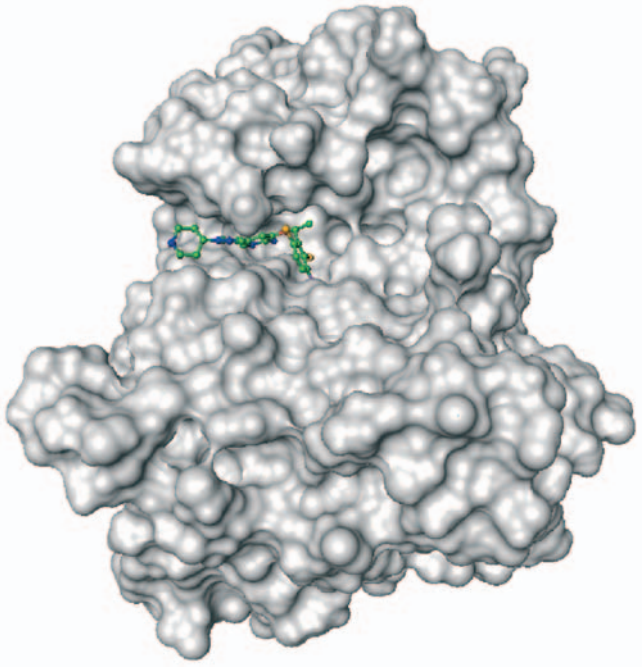

Figure 1. Crizotinib in the anaplastic lymphoma kinase ATP binding pocket [Camidge *et al.* 2010a].

Detection methods for *ALK* fusion genes

The optimum detection method for *ALK* has yet to be established. The most common methods currently in use include break-apart fluorescence *in situ* hybridization (FISH), immunohistochemistry (IHC), and reverse transcription polymerase chain reaction (RT-PCR). Break-apart FISH has to date been the usual standard method for confirmation of *ALK* status in clinical trials [Yi *et al.* 2011; Kwak *et al.* 2010].

Break-apart FISH employs a green centromeric probe and a red telomeric probe which bind to regions of the chromosome flanking the *ALK* gene. Thus, if the gene is split, as in the *EML4–ALK* fusion gene, the red and green signals will be separated; if not, a yellow signal will be seen. The assay is considered positive if fusion is detected in 15% or more of cells in the sample [Shaw *et al.* 2009]. However, break-apart FISH may require an experienced operator and this technique may not be widely available [Horn and Pao, 2009]. Fusion FISH can also be performed, using specific probes for the *EML4* and *ALK* genes [Sakairi *et al.* 2010].

IHC is a ubiquitous technique, in which antibodies labeled with markers are used to localize specific antigens in tissue samples. Detection with IHC can be difficult when the target antigen is expressed at low levels (as with ALK), and intermediate levels of staining may be open to interpretation. Furthermore, results can also be influenced by the way in which the sample is prepared and the detection system used [Koh *et al.* 2011; Ikeda *et al.* 2010; Takeuchi *et al.* 2009].

RT-PCR is a variant of PCR, in which reverse transcriptase is used to convert an RNA sequence into cDNA, which is then amplified using PCR [Bustin, 2000]. This technique provides an accurate indication of the presence of *ALK* abnormalities, and can be readily automated, making it suitable for high-throughput screening [Hirsch *et al.* 2010]. However, it requires predefined primers raised against known fusion genes, and hence previously unrecognized fusion partners may not be detected [Hirsch *et al.* 2010]. RT-PCR is technically difficult using formalin-fixed paraffin-embedded samples, although it may be feasible following recent advances in methodology [Danenberg *et al.* 2010; Hirsch *et al.* 2010; Mano, 2008].

Notably in this context, the chromosomal inversion leading to the formation of the *EML4–ALK* fusion gene does not always take place in the same location, and multiple *EML4–ALK* variants have been identified. All involve the tyrosine kinase domain of ALK, but there is variation in the truncation of *EML4*. At least 11 variants have been identified (see above), most of which are oncogenic [Sasaki *et al.* 2010b]. For example, the NSCLC cell lines H3122 and DFCI032 contain variants with fusions at *EML4* exon 13, whereas H2228 has a variant fused at *EML4* exon 6a/b [Koivunen *et al.* 2008]. In addition, as mentioned briefly earlier, *EML4* is not the only fusion partner for *ALK* in ALK-translocated NSCLC: *TFG* and *KIF5B* have also been identified in NSCLC tumor samples [Takeuchi *et al.* 2009; Rikova *et al.* 2007]. These variations in *EML4* fusion and the presence of non-*EML4* fusion partners for *ALK* carry implications for clinical testing and characterization of *ALK*-translocated NSCLC.

Treatment paradigm and screening

Two groups have investigated the correlation between IHC and FISH detection results as a way to facilitate ALK screening [Paik *et al.* 2011; Yi *et al.* 2011]. Both groups found 100% correlation between IHC score 0 and FISH negativity, and between IHC score 3+ and FISH positivity. Both studies concluded that samples with IHC scores of 1+ and 2+ would require confirmation by FISH for a reliable assessment of *ALK* status. Any practically useful screening process would need to demonstrate a low rate of false negatives, low variability in sensitivity and specificity of the antibody used, and robust reliability across multiple users and sites. Furthermore, such a system would need to retain flexibility to identify 'atypical' FISH-negative cases which may be positive using other screening techniques, and allow for FISH testing based on clinical suspicion [Camidge *et al.* 2011]. The use of IHC for ALK screening is desirable due to its practicality and wide availability, and further research into the use of this diagnostic technique is ongoing.

The challenge of finding an effective and widely implementable screening method for the *ALK* fusion gene is representative of the challenges facing the widespread implementation of personalized medicine in NSCLC. In order to ensure that patients receive the most appropriate treatment for their tumors, a robust and locally

available screening system is required. Such a system must not only provide detection of *EGFR*, *KRAS*, *ALK*, and any other aberration that influences treatment choice, but also do so quickly enough to give patients the best chance of a successful outcome from treatment; the collection of sufficient tissue for the various required tests should therefore be standard procedure [Hirsch *et al.* 2010]. The implementation of a successful screening system will therefore necessitate the close coordination of clinical, surgical, and pathology services.

Prevalence of *ALK* fusion genes in NSCLC

The *ALK* fusion gene has been reported to be present in up to 11.6% of patients with NSCLC [Zhang *et al.* 2010], depending on the population studied and screening methods used. The average prevalence appears to be approximately 3% in unselected populations and 4.5% in populations that have been 'enriched' by selection of patients with adenocarcinoma (Table 1).

Clinicopathological studies have identified patients with *ALK*-positive NSCLC as clinically distinct from those with *EGFR*-positive (based on age) and *EGFR* wild-type (based on age, smoking status, and tumor differentiation/grade) disease [Koh *et al.* 2011; Takahashi *et al.* 2010; Rodig *et al.* 2009; Shaw *et al.* 2009; Wong *et al.* 2009]. The *ALK* fusion gene appears to be more common in patients who have never smoked, or those who are light smokers, than in people who smoke [Zhang *et al.* 2010; Rodig *et al.* 2009; Wong *et al.* 2009]. For example, in a study of 266 NSCLC tumors taken from Chinese patients, the *ALK* fusion gene was present in 8.5% of tumors from patients who had never smoked compared with only 0.8% of tumors from patients who had smoked at some time during their lives [Wong *et al.* 2009]: a statistically significant difference ($p = 0.009$). Similarly, in a US study, 70% of patients with *ALK*-positive NSCLC had never smoked [Rodig *et al.* 2009]. Again, this association was highly statistically significant ($p < 0.0001$; $N = 358$). Patients with *ALK*-positive disease also tend to be younger than those with *ALK*-negative disease. In a study by Koh and colleagues, patients with *ALK*-positive disease were significantly younger than those with *ALK*-negative disease, with a median age of 49 years compared with 61 years ($p < 0.001$; $N = 221$) [Koh *et al.* 2011]. Similarly, the median age of patients with *ALK*-positive NSCLC in the US study above was 51 years, *versus* 66 years in those with *ALK*-negative disease ($p = 0.0002$ for the difference; $N = 358$) [Rodig *et al.* 2009]. In addition to age, Koh and colleagues found that signet ring cell components were frequently observed ($p = 0.056$, $N = 221$) in *ALK*-positive tumors and TTF-1 expression was observed in all *ALK*-positive tumors for which IHC data were available [Koh *et al.* 2011]. In addition, *ALK* status of patients with NSCLC does not seem to influence response to platinum-based doublet chemotherapy [Koh *et al.* 2011; Shaw *et al.* 2009].

Table 1. Prevalence of *anaplastic lymphoma kinase* (*ALK*) fusion gene in unselected and adenocarcinoma-enriched patient populations with non-small cell lung cancer.

Study	Country	Unselected populations		Adenocarcinoma-enriched populations	
		Total, *n*	*ALK*-positive, *n* (%)	Total, *n*	*ALK*-positive, *n* (%)
[Soda *et al.* 2007]	Japan	75	5 (6.7)		
[Rikova *et al.* 2007]	China	103	4 (3.9)		
[Koivunen *et al.* 2008]	USA/Korea	305	8 (2.6)	200	8 (4.0)
[Inamura *et al.* 2008]	Japan	221	5 (2.3)	149	5 (3.4)
[Takeuchi *et al.* 2008]	Japan	343	11 (3.2)	253	11 (4.3)
[Perner *et al.* 2008]	USA/Switzerland	603	16 (2.7)		
[Shinmura *et al.* 2008]	Japan	77	2 (2.6)		
[Boland *et al.* 2009]	USA	335	6 (1.8)	185	5 (2.7)
[Wong *et al.* 2009]	China	266	13 (4.9)	209	11 (5.3)
[Martelli *et al.* 2009]	EU	120	9 (7.5)	63	3 (4.8)
[Rodig *et al.* 2009]	USA			358	20 (5.6)
[Takeuchi *et al.* 2009]	Japan			130	4 (3.1)
[Takahashi *et al.* 2010]	Japan	313	5 (1.6)	211	5 (2.4)
[Zhang *et al.* 2010]	China	103	12 (11.7)	62	10 (16.1)
Total		2864	96 (3.4)	1820	82 (4.5)

To date, data suggest that the *ALK* fusion gene appears to be more common in younger patients, patients who have never smoked, and patients with adenocarcinoma. However, the various published studies to date are subject to their own limitations with the consequent possibility of selection bias and, in lieu of a comprehensive study, we must consider that any patient with NSCLC can carry the *ALK* fusion gene. For example, the literature includes a case of *ALK*-positive NSCLC in an elderly patient (aged 76 years) with a history of smoking [Rodig *et al.* 2009]. Clinical characteristics alone are therefore not sufficient to identify patients with *ALK*-positive disease [Rodig *et al.* 2009]. Further studies are needed to better understand the clinical characteristics of patients with *ALK*-positive disease.

Interestingly, a consistent finding has been that the *ALK* fusion gene is largely exclusive of *EGFR* and *KRAS* mutations: in other words, patients with this fusion gene seldom have concomitant mutations in *EGFR* or *KRAS* [Sun *et al.* 2010; Yoshida *et al.* 2010; Zhang *et al.* 2010; Camidge *et al.* 2010b; Rodig *et al.* 2009; Shaw *et al.* 2009], and patients with *EGFR* or *KRAS* mutations do not generally have concurrent *EML4–ALK* rearrangement, although comutation with *EGFR* has been reported [Tiseo *et al.* 2011]. Also, *ALK* fusion is not limited to adenocarcinoma, having been reported in patients with squamous cell lung carcinoma histology [Boland *et al.* 2009; Wong *et al.* 2009; Soda *et al.* 2007].

Clinical data for crizotinib in NSCLC

Current clinical data supporting the efficacy of crizotinib in patients with *ALK*-positive NSCLC come from an expanded cohort study (A8081001 [ClinicalTrials.gov identifier: NCT00585195]), which enrolled patients with *ALK*-positive disease regardless of prior therapy [Kwak *et al.* 2010]. *ALK* positivity was confirmed by break-apart FISH in all cases. Patients received crizotinib 250 mg twice daily continuously. Data from the first 82 patients with NSCLC were presented at the 2010 American Society of Clinical Oncology Annual Meeting [Bang *et al.* 2010] and subsequently reported in the *New England Journal of Medicine* [Kwak *et al.* 2010]. An update to these data with 113 enrolled patients and a median follow up of 8 months was presented at the 2010 European Society of Medical Oncology Congress [Camidge *et al.* 2010a]. Patients were younger than is typical for patients with NSCLC, tended to have never smoked, and usually had adenocarcinoma tumors (Table 2). As of August 2010, there were 105 patients evaluable for response, including two complete responses and 57 partial responses (objective response rate 56%), plus a further 33 patients (31%) with stable disease [Solomon *et al.* 2010]. The best reduction in tumor size is shown in Figure 2, from which it can be seen that most patients showed a decrease from baseline in target lesion tumor size of 30% or more. Responses to crizotinib occurred quickly, with 56% of responses occurring by week 8 of treatment (Figure 3) [Solomon *et al.* 2010]. Median PFS was 9.2 months (95% CI 7.6 to 10.3; Figure 4), with a probability of being progression free at 6 months of 71.3% (95% CI 60.3 to 79.7) [Camidge *et al.* 2010a].

Table 2. Baseline characteristics of patients with non-small cell lung cancer participating in a study of crizotinib ($N = 113$) [Camidge *et al.* 2010a].

Characteristic	
Number of patients	113
Mean age (range), years	52 (21–79)
Gender (male/female), *n* (%)	57/56 (50.4/49.6)
ECOG performance status, *n* (%)	
0	38 (33.6)
1	60 (53.1)
2	15 (13.3)
Ethnicity, *n* (%)	
White	69 (61.1)
Asian	34 (30.1)
Other	10 (8.8)
Smoking history, *n* (%)	
Never	82 (72.6)
Former	30 (26.5)
Current	1 (<1.0)
Histology, *n* (%)	
Adenocarcinoma	109 (96.5)
Squamous cell carcinoma	1 (<1.0)
Large cell	1 (<1.0)
Other	2 (1.8)
Previous treatment regimens, *n* (%)	
0	6 (5.3)
1	32 (28.3)
2	21 (18.6)
3	17 (15.0)
>3	35 (31.0)
Not reported	2 (1.8)

ECOG, Eastern Cooperative Oncology Group.

The A8081001 protocol made a provision for patients to continue to receive crizotinib after Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0-defined disease

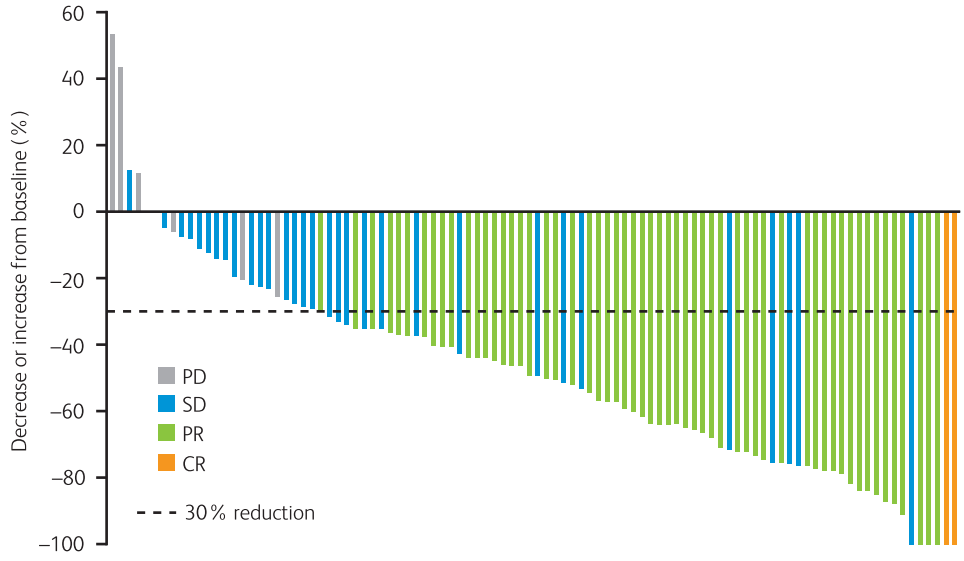

Figure 2. Waterfall plot of best percentage change from baseline in target lesions by patient in patients with *anaplastic lymphoma kinase (ALK)*-positive non-small cell lung cancer who received crizotinib (excluding those with early death and indeterminate or unavailable response from the 105 evaluable patients; August 2010) [Camidge *et al.* 2010a]. CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.

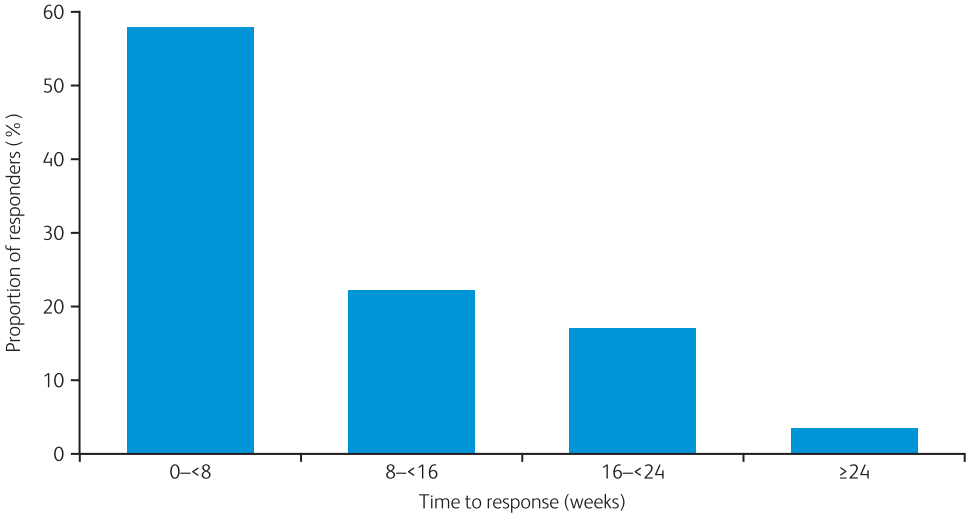

Figure 3. Time to response with crizotinib for responding patients ($n = 59$ of 105 evaluable patients; August 2010) [Solomon *et al.* 2010].

progression if the investigator felt that the patient would derive clinical benefit. Interestingly, of 36 patients who experienced RECIST-defined progression, 15 received postprogression crizotinib for more than 2 weeks and five received crizotinib for longer than 6 months [Camidge *et al.* 2010a].

The most common treatment-related adverse events (AEs) in this analysis (August 2010; $N = 113$) were grade 1/2 gastrointestinal AEs (nausea, 52%; diarrhea, 50%; vomiting, 42%; all were manageable with standard antiemetic or antidiarrheal treatments) and grade 1 visual

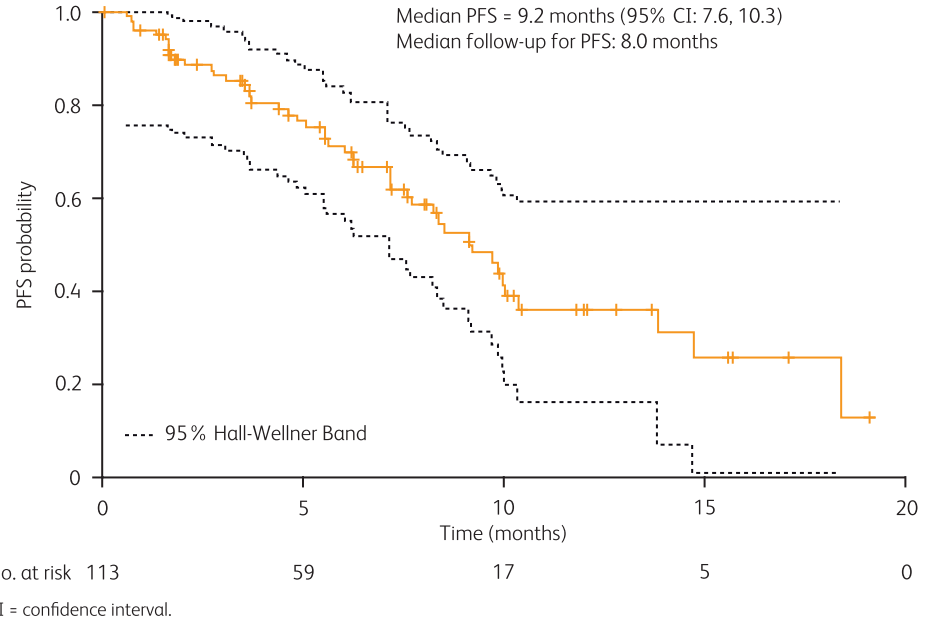

Figure 4. Progression-free survival (PFS) following treatment with crizotinib (August 2010; $N = 113$) [Camidge *et al.* 2010a]. CI, confidence interval.

disturbance in 45% of patients (Table 3) [Camidge *et al.* 2010a]. Visual disturbances with crizotinib have been described as 'trails of light' when accommodating from dark to light [Clinical Care Options 2010; Kwak *et al.* 2010]. Grade 3/4 treatment-related AEs included increases in alanine aminotransferase (4% grade 3; 1% grade 4) and aspartate aminotransferase (4% grade 3). Neutropenia and lymphopenia were also reported at grade 3 severity (3% and 2% respectively). One patient experienced grade 3 pneumonitis, but this was not considered to be related to treatment [Camidge *et al.* 2010a]. A total of 36 patients (31.9%) had discontinued study treatment by August 2010 (median follow up 8 months; progressive disease, $n = 23$; death, $n = 7$ unrelated to treatment; AEs, $n = 3$ with one considered treatment related; withdrawal of consent, $n = 1$; and other, $n = 2$) [Camidge *et al.* 2010a].

Data indicate differences in the pharmacokinetics of crizotinib between Asian and non-Asian patients, with body weight and surface area accounting partially but not completely for the observed pharmacokinetic differences [Ou *et al.* 2010]. Steady-state mean area under the curve of crizotinib, adjusted for body weight and adjusted for body surface area, was found to be 26% and 46% higher respectively in Asian patients compared with non-Asian patients. A higher overall response rate to crizotinib was also seen in Asian patients, although clinically significant responses to crizotinib were also seen across all ethnicities. As such, current efficacy and safety results support clinical investigation of crizotinib with the same dose regimen in Asian and non-Asian patients.

Future research and clinical development for crizotinib and NSCLC

At this early stage in the development of crizotinib, there are several issues which have still to be fully explored. As with all targeted cancer therapies, resistance to crizotinib is likely to be a significant issue for therapy, and there is already a recorded case of crizotinib resistance in a young patient with *EML4–ALK*-positive NSCLC [Choi *et al.* 2010]. Two independent mutations were identified; a substitution of adenine for guanine at position 4374 of *EML4–ALK*, resulting in replacement of cysteine with tyrosine at position 1156 of *ALK* (C1156Y), and a substitution of adenine for cytosine at *ALK* position 4493, resulting in replacement of leucine with methionine at position 1196 of *ALK* (L1196M) [Choi *et al.* 2010]. A third mutation (F1174L) has been identified in a patient with *RANBP2–ALK*-positive inflammatory myofibroblastic tumor, and was associated with decreased sensitivity of

Table 3. Most common treatment-related adverse events (≥10%) with crizotinib ($N = 113$) [Camidge *et al.* 2010a].

Adverse event	Grade 1, *n* (%)	Grade 2, *n* (%)	Grade 3, *n* (%)	Grade 4, *n* (%)	Total, *n* (%)
Nausea	58 (51.3)	1 (0.9)	0	0	59 (52.2)
Diarrhea	55 (48.7)	2 (1.8)	0	0	57 (50.4)
Visual impairment	51 (45.1)	0	0	0	51 (45.1)
Vomiting	46 (40.7)	1 (0.9)	0	0	47 (41.6)
Constipation	22 (19.5)	6 (5.3)	0	0	28 (24.8)
Peripheral edema	19 (16.8)	3 (2.7)	0	0	22 (19.5)
Decreased appetite	21 (18.6)	0	0	0	21 (18.6)
Dizziness	21 (18.6)	0	0	0	21 (18.6)
Fatigue	14 (12.4)	3 (2.7)	1 (0.9)	0	18 (15.9)
Alanine aminotransferase increased	3 (2.7)	6 (5.3)	4 (3.5)	1 (0.9)	14 (12.4)

Ba/F3 cells to crizotinib, although this mutation was unlikely to directly prevent binding of crizotinib to ALK [Sasaki *et al.* 2010a]. Further investigation of mechanisms of resistance to crizotinib and how to overcome it will be crucial [Butrynski *et al.* 2010]. The potential of combination therapy with different intracellular signaling inhibitors to target proliferation and resistance pathways simultaneously is also an option, as is the development of other agents to overcome crizotinib resistance.

As research into oncogenic drivers in NSCLC continues, it is inevitable that new molecular targets will be discovered for distinct patient subgroups. Experience with crizotinib shows that it is feasible to develop agents for new targets in NSCLC and subsequently progress to clinical trials within a relatively short timeframe. It should also be noted that crizotinib may show promise in other cancers harboring *ALK* gene rearrangements such as anaplastic large-cell lymphomas [Gambacorti-Passerini *et al.* 2011]. In summary, the integration of crizotinib and of future personalized therapies into standard treatment practice in NSCLC will rest on the widespread implementation of an effective screening system for newly diagnosed patients with NSCLC which is flexible enough to incorporate new targets as treatments are developed for them.

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Dr Bang has performed a consultation/advisory role for Pfizer Inc.

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