The therapeutic potential of anaplastic lymphoma kinase inhibitors in lung cancer: rationale and clinical evidence

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In the last few years it has been demonstrated that approximately 4% of non-small-cell lung cancer is driven by an *ALK* gene rearrangement. When such a rearrangement occurs an oncogene addicted state appears to then exist within the cancer cell. Crizotinib, an anaplastic lymphoma kinase (ALK) tyrosine kinase inhibitor, is effective in preclinical models of *ALK*-rearranged lung cancer, and has demonstrated high response rates in Phase I/II clinical trials in this sub-population. This review summarizes ALK biology, recent developments in techniques for the detection of *ALK* rearrangements, clinical characteristics of this population and relevant clinical trial results. Following filing for accelerated approval, crizotinib may soon be licensed for the treatment of ALK-positive non-small-cell lung cancer in the USA as well as worldwide.

Keywords: ALK gene rearrangement • break-apart FISH • crizotinib • EML4-ALK • L1196M gate keeper mutation

Until recently, patients with metastatic non-small-cell lung cancer (NSCLC) were treated as a largely homogenous group with respect to which systemic palliative cytotoxic chemotherapy to choose. However, over the last ten years, clinically relevant histological and, increasingly, molecular differences between NSCLC cases are being recognized. On a molecular level, somatic mutations within the EGFR gene in NSCLC represented the first example of a testable molecular abnormality in the tumor that dominantly affected sensitivity to a specific pharmacological intervention. Tumors with mutations in either exons 19 or 21 of the EGFR are often exquisitely sensitive to the EGFR tyrosine kinase inhibitors (erlotinib and gefitinib), leading to high response rates, prolonged progression-free survival (PFS), and improved quality of life relative to first line cytotoxic chemotherapy [1-3]. Now, another clinically significant molecular subgroup of NSCLC has been identified: tumors with activating rearrangements of the ALK gene. Patients with ALK gene rearrangements can derive significant benefit in terms of tumor shrinkage following treated with crizotinib, an orally bioavailable small molecule inhibitor of the anaplastic lymphoma kinase (ALK) tyrosine kinase [4]. In 2011, Pfizer, the manufacturers of crizotinib filed with the US FDA seeking accelerated approval of their drug in ALK-gene rearranged NSCLC. Herein we highlight this novel subset of NSCLC, exploring both the methods of detecting ALK-positive lung cancer and the licensed and experimental therapeutic options that may be available to patients with this disease.

ALK biology

While the physiological role of native ALK in adult humans has still be to defined, studies in nonhuman species suggests that it may have a role in the development of both the gut and the visual system [5]. However, oncogenic activation of ALK has





been described in a number of different human cancers, including anaplastic large-cell lymphomas (ALCL) and inflammatory myofibroblastic tumors [5-15]. In most cases, activation is through a transforming rearrangement that places one of several different 5' fusion partners (in conjunction with their associated promoter region) upstream of the region encoding the 3' kinase domain of ALK[5,12]. Transforming rearrangements of ALK were first identified through cloning of the t(2;5) p23;q35) translocation found in a subset of ALCL [13]. In 2007, a Japanese group identified an ALK gene rearrangement in NSCLC by looking for transforming transcripts from a retroviral cDNA expression library derived from the tumor of a patient with NSCLC who was known to be both EGFR and KRAS wildtype [16]. The transforming gene fusion they identified resulted from an inversion on the short arm of chromosome two that juxtaposed the 5' end of the EML4 gene with the 3' end of the ALK gene, encoding a novel chimeric protein with constitutive activation of the intracellular portion of ALK [16]. The 5' EML4 partner is variably truncated, although all variants still include the coiled coil domain of the protein necessary for the ligand independent dimerization or oligomerization of the ALK tyrosine kinase [17,18]. Less commonly in NSCLC, constitutive activation of the ALK tyrosine kinase can result from rearrangement with genes other than EML4, such as TFG and KIF5B [19,20]. The cellular consequences of activation of the ALK tyrosine kinase are subsequent signaling through the phospholipase y, PI3K, RAS/MAPK and JAK/STAT pathways [5,21]. Cells transformed through ALK gene rearrangements appear to be highly sensitive to ALK inhibition consistent with the development of an 'oncogenic addiction' [18,22].

Clinical & histological features of *ALK* gene-rearranged lung tumors

The *ALK* rearrangement occurs in approximately 4% of NSCLC [18,23–29]. Identifying specific clinical and pathological features associated with the presence of this molecular abnormality may help to focus screening techniques to increase both the hit-rate and the cost effectiveness of screening. However, unless groups with a zero percent chance of ALK positivity can be identified, all other enrichment strategies will inevitably exclude some patients from a potentially highly appropriate treatment if their chances of positivity are viewed as 'too low' to recommend screening. What an appropriate to screen for *ALK* rearrangements is a debate that has barely started in thoracic oncology.

The strongest feature correlated with *ALK* rearrangements is the fact that they are usually mutually exclusive with EGFR and *KRAS* mutations, although

exceptions do occur [16,24,25,28-30]. This is presumably because there is little evolutionary selection pressure for two primary oncogenic drivers to occur in the same disease at the same time in the untreated state. ALK rearrangements are mostly found among tumors with adenocarcinoma histology (58/1511; 3.8%), but they have also been found among tumors with non-adenocarcinoma histology (7/579; 1.2%) (Table 1) [18,23-29]. Given that pathologists may not always agree on a histological classification, with disagreement rates between the categorization of adenocarcinoma versus non-adenocarcinoma histology as high as 10-20% [31-33], the lack of a complete association between a molecular marker and a single histology in NSCLC is perhaps not surprising. Therefore exclusively testing NSCLC with adenocarcinoma histology for ALK rearrangements may miss approximately 10% of such abnormalities (Table 1). In general, patients with ALK rearrangements tend to be never or light smokers [24,30,34], although ever smokers make up approximately 44% of patients with ALK rearrangements (Table 2), and a history of smoking should probably not prejudice a practitioner against testing for ALK rearrangements in any given NSCLC patient. The average age of patients with ALK rearrangements tends to be lower than those without such changes, but the range of ages affected is very broad (Table 3) [25,30,35,36]. Discrepancies between currently published series on matters such as the proportion of NSCLC tumors with ALK rearrangements, differences in median age and prognosis may be explained both by the small sizes of the positive groups in most series and by the fact that some series reflect analyses conducted on enriched populations [30,35] compared with unselected series [25,36]. Several different histological patterns have been reported to be particularly associated with ALK gene rearrangements in adenocarcinoma such as signet-ring histology with abundant intracellular mucin and poor differentiation with an acinar pattern [24,30]. However, the reliability of these associations across multiple different observers and centers and their overall usefulness with respect to testing strategies remains to be determined.

Detecting ALK gene rearrangements

Techniques to identify the presence of ALK rearrangements include FISH, immunohistochemistry (IHC), and polymerase chain reaction (PCR) based methods. Of these, break-apart FISH is currently the gold standard, reflecting the availability of a commercial probe set (Abbott Molecular, Abbott Park, IL, USA), previously developed for diagnosis of ALCL. The green (5') and red (3') fluorescent probes bind to areas upstream and downstream of the common breakpoint in the ALK

screening of sequential tumor specimens.					
Author	Ethnicity	Adenocarcinoma, ALK+ no.	Non-adenocarcinoma, ALK+ no.	Ref.	
Varella-Garcia et al.	Italian	12/315	0/138	[28]	
Boland <i>et al</i> .	American	5/185	1/150	[23]	
Martelli <i>et al</i> .	European	5/65+	4/48	[25]	
Koivunen <i>et al</i> .	Korean/American	8/217+	0/88	[18]	
Wong et al.	Hong Kong	11/209	2/57	[29]	
Shinmura <i>et al</i> .	Japanese	2/53*	0/24	[27]	
Inamura <i>et al</i> .	Japanese	5/149 ⁺	0/48	[24]	
Mitsudomi et al.	Japanese	10/318+	0/26	[26]	
Total		58/1511 (3.8%)	7/579 (1.2%)		
Proportion of <i>ALK</i> rearrangements by histology		58/65 (89%)	7/65 (11%)		
*Series includes tumors with adeno-squamous histology.					

Table 1. Published series of the frequency of *ALK* rearrangements that include both adenocarcinoma and non-adenocarcinoma (squamous, large-cell but not small-cell carcinoma) from unselected screening of sequential tumor specimens.

gene, respectively. In normal cells, the native signal appears as a fused red and green (yellow) fluorescent pattern. When ALK rearrangement occurs, independent of its fusion partner, the red and green signals separate and distinct red and green signals are seen (Figure 1). A positive signal is defined if more than 15% of scored tumor cells have split signals or solitary red (3') signals when examining 4+ fields (~60 cells) [4,35]. The Abbott Molecular break-apart FISH assay has been used as the entry diagnostic test for all of the clinical trials of crizotinib to date (see below), and therefore is considered the gold standard for ALK positivity [37]. Commercial providers of ALK testing, beyond those used for the crizotinib trials, are becoming increasingly common, often offering FISH testing using exactly the same probe sets. However, FISH interpretation requires an expert lab to minimize both false positive and negative cell counts, which can occur due to subjective assessment

of the distance between the breakapart probes. In addition, the exact criteria utilized within the trials for calling an individual cell positive (split or single red signals; but, currently, not *ALK* copy number alone, and not single green signals alone) and for calling an individual tumor positive (>15% cells positive with at least 50 nuclei counted) are not always strictly adhered to by some providers. Using the break-apart FISH assay, positive results are correctly referred to as *ALK* gene rearrangements or *ALK* fusions. A more detailed description of the positivity, for example, referring to it as *EML4-ALK* positive is not technically possible as the 5' fusion partner is not determined with this test.

Reverse transcriptase (RT)-PCR based techniques for detecting *ALK* rearrangements also exist [38]. Several are, or will become, commercially available. These assays may offer the potential benefit of increased sensitivity, and the ability to identify the exact fusion partner as well as the ability to distinguish different breakpoints in the same 5' part nerby using multiplex PCR techniques [39]. Most academic assays require fresh or frozen tissue, due to some degradation of RNA extracted from formalin-fixed paraffin tissue. Newer PCR techniques being commercially developed may overcome such limitations and allow accurate assessments from formalinfixed paraffin-embedded tissue [39]. Of note, RT-PCR is highly specific and some fusion partners or breakpoint

Table 2. Published series of <i>ALK</i> rearrangements that have separated never smokers	
from ever smokers (including light or heavy smokers).	

Author	Ethnicity	Nonsmoker, ALK+ no.	Smoker, ALK+ no.	Ref.
Martelli <i>et al</i> .	European	1	8	[25]
Koivunen <i>et al.</i>	Korean/American	2	4	[18]
Wong et al.	Hong Kong	12	1	[29]
Shinmura <i>et al</i> .	Japanese	0	2	[27]
Inamura <i>et al</i> .	Japanese	3	2	[24]
Mitsudomi et al.	Japanese	7	3	[26]
Proportion of ALK r smoking status	earrangements by	24/45 (56%)	20/45 (44%)	

Review: Clinical Trial Outcomes Weickhardt & Camidge

Table 3. Age and <i>ALK</i> rearrangements.			
Author	Median age (years)	Range of age (years)	Ref.
Shaw et al.	52	29–76	[30]
Camidge <i>et al</i> .	53	34–75	[35]
Jokoji <i>et al</i> .	66	44–77	[36]
Martelli <i>et al</i> .	64	54–85	[25]

variants may be missed if there are not primers present to specifically look for these. When the *ALK*FISH positive cases were retrospectively analysed for *EML4-ALK* transcripts by RT-PCR in the initial Phase I study of crizotinib, transcripts could not be detected in 31% of cases [4]. While some of these RT-PCR negative cases could represent a failure of technique, they could also represent non-*EML4* fusion variants that were being missed by the highly specific *EML4-ALK*RT-PCR that was being conducted.

IHC analysis for ALK is a third method of detection, with a variety of antibodies in commercial development (including antibodies supplied by Cell Signaling, Novacastraand DAKO) [40-42]. While earlier approaches using IHC had inadequate sensitivity for ALK detection as correlated with FISH [43], newer techniques have a reported sensitivity as high as 100% [41]. However, specificity is still highly variable. Since specificity is not always 100%, the possibility of using IHC as a screening test prior to FISH confirmation has been raised [41,42]. In the study by Paik *et al.* performed on a tissue microarray using the Novacastra antibody [41], 92% (603/652) of patients lacked ALK IHC expression and none of these patients were ALK FISH positive. Of the 8% (49/653) with at least 1+ expression, only 45% (22/49) were FISH positive. Whilst attractive as a method for potentially minimizing the utilization of FISH testing – which is less freely available than IHC – the ideal antibody to use, and the reliability of IHC across multiple labs and multiple observers, and the cost of making IHC this reliable relative to FISH, all currently remain unclear [44].

Clinical studies of ALK inhibition

Xenograft models derived from *EML4-ALK* NSCLC cell lines have demonstrated that ALK inhibition with a small molecule tyrosine kinase inhibitor leads to effective and prolonged tumor regression [16,45]. PF-02341066 (crizotinib) is a small molecule inhibitor of the ALK tyrosine kinase (IC_{50} : 20 nM) and of mesenchymal epithelial transition receptor (c-MET)(IC50 8 nM). Whilst crizotinib is the only ALK inhibitor currently with published clinical data, several other ALK inhibitors have entered or are due to enter Phase I/II testing soon (Table 4).

The initial Phase I trial of crizotinib (A8081001; NCT00585195) began in 2006, with planned dose expansion cohorts in patients with proven evidence of either ALK or MET activation based on published preclinical data suggesting these would be highly sensitive populations [16,46]. Data from the *ALK* gene rearranged NSCLC expanded cohort was published in 2010, demonstrating a high response rate of 56% and median PFS of 9.2 months in 115 FISH-positive *ALK* rearranged NSCLC patients [4].



Figure 1. Non-small-cell lung cancers hybridized with the *ALK* break-apart FISH probe (Abbott Molecular, Abbott Park, IL, USA). Native *ALK* status (indicated by yellow arrows) shows fusion of the probes adjacent to the 3' and 5' ends of the gene, labeled, respectively, with red and green fluorophores. Rearranged *ALK* is indicated by the presence of split 3' (red arrows) and 5' (green arrows) signals, including single red signals. **(A)** *ALK* FISH-negative specimen. **(B)** 'Classic' *ALK* FISH-positive specimen showing split red and green signals. **(C)** *ALK* FISH-positive specimen showing two or three copies of single red signals per cell. **(D)** *ALK* FISH-positive specimen showing evidence of gene copy number increase with clusters of multiple single red and single green signals (double arrows).

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Table 4. Anaplastic lymphoma kinase inhibitors currently in development.				
Drug	Company	Phase of testing	Status	Clinicaltrials.gov ID
Crizotinib (PF-023341066)	Pfizer	Phase II/III	Open	NCT00585195, NCT00932893, NCT01154140 and NCT00932451
ASP-3026	Astellas	Phase I	Open	NCT01284192
XL228	Elexis	Phase I	Completed	NCT00526838
LDK378	Novartis	Phase I	Open	NCT01283516
AP-26113	Ariad	Preclinical		
CH5424802	Chugai	Preclinical		
CEP-37440	Cephalon	Preclinical		
Data taken from [101].				

Crizotinib so far seems to be well tolerated with few serious grade 3/4 adverse events aside from reversible drug induced transaminitis, which was reported in 6 out of 105 patients in the initial trial. Grade 1–2 gastrointestinal toxicities including nausea (52%), diarrhea (46%) and vomiting (43%) were the most common adverse events [4]. Interestingly, a novel adverse event experienced by 42% of patients with crizotinib in the initial study was transient visual disturbance, which has been described as trails of lights in peripheral vision in low light conditions. No patients discontinued therapy because of adverse events in the clinical trial [4].

On the basis of these encouraging results, a randomized Phase III trial of crizotinib has now started (PROFILE-1007). In this study crizotinib is compared with standard second line chemotherapy (either docetaxel or pemetrexed) in patients determined as ALK positive using the Abbott Molecular FISH assay with break-apart probes performed by a central laboratory (NCT00932893). A multicenter randomized Phase III trial (PROFILE 1014 and NCT01154140) is also open for ALK-positive patients in the first-line setting comparing crizotinib with a platinum/pemetrexed doublet. Patients ineligible for either trial or who are randomized to receive standard chemotherapy in PROFILE 1007 and then progress, may be eligible to receive crizotinib in a companion single arm Phase II trial (PROFILE 1005 and NCT00932451). Pfizer has now filed for accelerated approval for crizotinib on the basis of safety and efficacy from the Phase I study, and interim data from the ongoing single-arm PROFILE-1005 trial. A decision is expected in the second half of 2011.

Resistance to ALK inhibitors

Despite high response rates, 50% of patients still progressed within 10 months of commencing therapy with crizotinib [4]. Possible mechanisms of acquired resistance continue to be explored. Using an accelerated *in vitro* mutagenesis screen several mutations in ALK including the L1196M gate keeper mutation that confers resistance to crizotinib, analogous to T790M mutations in the EGFR occurring with EGFR-TKI therapy have been identified. In 2010, a single case demonstrating the clinical achievability of such acquired resistance mutations was reported. Two acquired mutations, C1156Y and L1196M, were identified in the same Japanese patient with ALK rearranged NSCLC following progression after initial response to crizotinib [47]. Other resistance mechanisms undoubtedly also exist and remain to be described. Several second generation ALK inhibitors appear to have sufficiently high binding affinities that they may be able to overcome treatment resistance that is due to some of these acquired ALK mutations (Table 4) [48]. However, until we know the exposures of these novel agents that are clinically achievable, and more importantly the frequency with which these acquired ALK mutations might occur at, we will not know the true potential of these novel agents in this patient population.

Other drugs with potential activity in *ALK* gene-rearranged NSCLC

Patients with ALK rearrangements appear to derive little benefit from the use of EGFR-TKI therapy [30]. They appear to have similar outcomes to patients with adenocarcinoma without such rearrangements when treated with platinum based chemotherapy [30]. However, patients with ALK rearrangements have significantly longer PFS when treated with pemetrexed-based regimens to other molecularly categorized NSCLC groups [49,50]. The basis for this super-sensitivity to pemetrexed is unclear, but may relate to the activated ALK tyrosine kinase in the tumor increasing activity of folate pathway enzymes, sensitizing the tumor to cytotoxic chemotherapy targeting this pathway [50]. The significance of this early clinical observation to the development plans for crizotinib that involve comparisons to pemetrexed containing regimens within randomized studies remains to be seen. Similarly, whether pemetrexed will retain this potency in the crizotinib resistant setting is also unknown. Recently, experimental heat shock protien (HSP) 90 inhibitors have also shown some promise in ALK gene rearranged NSCLC [51]. Preclinical work has demonstrated that EML4-ALK is a sensitive client protein of HSP90 and ALK-transformed NSCLC cell lines are highly sensitive to HSP90 inhibition [52]. Intriguingly, in a Phase II trial of an HSP90 inhibitor in NSCLC, retrospective analysis showed that two out of five patients who responded to therapy had ALK rearrangements [51]. Of note, all of these patients were crizotinib naïve, and the potential of these agents in the crizotinib resistance setting will probably depend on the exact mechanism of resistance employed by an individual's tumor and whether ALK (and/or other HSP90 client proteins) remain the dominant drivers of the cancer cell.

Future perspective

Crizotinib is likely to be the first of many therapeutic options for ALK-rearranged NSCLC, a newly molecularly defined subtype of lung cancer. Better understanding will develop regarding the mechanisms of acquired resistance to crizotinib, which will further refine therapy for this population. The potential for differential benefit of molecularly defined groups from established therapies (pemetrexed, in the case of ALK-rearranged NSCLC) may significantly impact the choices of standard therapies for molecularly defined subgroups patients, and be needed to inform the most appropriate comparator chemotherapy to use in registration studies in the future. The rapid transition from Phase I to FDA filing for crizotinib, following the use of molecularly defined cohorts within early phase studies is likely to significantly influence drug development strategies for targeted agents in cancer in the coming years.

Summary

ALK gene rearrangements occur in approximately 4% of patients with NSCLC. Although several different detection methods are being explored, significant

clinical benefit from the use of crizotinib has only been prospectively explored among those proven to be ALK positive through the use of break-apart FISH testing. Only one ALK inhibitor, crizotinib, is significantly advanced in clinical development. On the basis of exceptional response rates and long PFS, accelerated approval of crizotinib in ALK-positive NSCLC is being sought from the FDA. If granted, this approval will likely be conditional upon the results of ongoing randomized studies comparing crizotinib to chemotherapy in this population. As with other targeted therapies given to sensitive populations, it is expected that all ALK-positive patients will eventually develop acquired resistance to crizotinib. Gatekeeper mutations in the ALK have already been described but other mechanisms of resistance are likely to occur. The potential for second generation ALK inhibitors to overcome this mechanism of resistance, and its overall expected clinical frequency remain unknown. Other agents, including pemetrexed and HSP90 inhibitors appear to have prominent activity in crizotinib-naïve ALK-gene rearranged NSCLC. Theirrole in crizotinibresistance cases remains to be elucidated. The rapid procession from discovery of ALK in NSCLC in 2007 to the publication of high rates of clinical activity of crizotinib has propelled the treatment of NSCLC forward significantly [4,16]. The rationale of directing the right drug to the right patient, through the use of prospective molecular profiling early in clinical development appears well validated and is likely to inform the development of many other targeted agents in oncology in the future.

Financial & competing interests disclosure

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Executive summary

Transforming rearrangements in anaplastic lymphoma kinase (ALK) occur in approximately 4% of non-small-cell lung cancer.

- ALK rearrangements lead to constitutive activation of the ALK tyrosine kinase.
- ALK rearrangements within all crizotinib trials to date have beendiagnosed by FISH, but alternate techniques such as polymerase chain reaction and immunohistochemistry exist.
- Crizotinib, an ALK inhibitor, has a high rate of clinical activity in patients with proven ALK rearrangements.
- Mechanisms of resistance to crizotinib are yet to be fully elucidated but acquired gate keeper mutations in ALK that reduce the cell's sensitivity to crizotinib have already been described.

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Review: Clinical Trial Outcomes Weickhardt & Camidge

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