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Treating ALK positive lung cancer: Early successes and coming challenges

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Abstract

Rearrangements of the anaplastic lymphoma kinase (ALK) gene occur infrequently in non-small cell lung cancer (NSCLC), but provide an important paradigm for oncogene-directed therapy in this disease. Crizotinib, an orally bioavailable inhibitor of ALK, provides significant benefit for patients with ALK positive (ALK+) NSCLC in association with characteristic, mostly mild, toxicities and is now FDA approved in this molecularly defined subgroup of lung cancer. Many new ALK inhibitors are being developed and understanding the challenges of determining and addressing the side-effects that are likely to be ALK specific, maximizing the time of benefit on targeted agents, and understanding the mechanisms that underlie drug resistance will be critical in informing the optimal therapy of ALK+NSCLC in the future.

Keywords

ALK; Lung cancer; crizotinib

Introduction

The Anaplastic Lymphoma Kinase (*ALK*) gene was first identified in its aberrant, disease-causing form. A reciprocal translocation between chromosomes 2 and 5 (t(2;5) (p23;q35)) had been noted in a subset of anaplastic large cell lymphomas (ALCL). [1] Later this was proven to generate a fusion gene combining the 5′ end of nucleophosmin (*NPM*) with the 3′ kinase encoding region of a novel gene, subsequently named *ALK*. [2] However, it is the potential of aberrant ALK to act as an oncogene in many different cancers, especially non-small cell lung cancer (NSCLC) that has heightened interest in ALK as a therapeutic target (Box 1). [3] In 2011, on the basis of dramatic and prolonged objective responses, the FDA

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Review criteria

The relevant articles were identified by the authors using PubMed (Searchterms: Anaplastic lymphoma kinase, ALK and lung) and on the basis of their knowledge of the biology of ALK+ disease and the clinical development of ALK inhibitors. The published data were inclusive of articles published from January 1994 through to January 2012, and abstracts from major conferences (including the Annual Meeting of ASCO and European Society of Medical Oncology from 2009 to 2011).

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approved crizotinib as the first licensed ALK inhibitor for ALK positive (ALK+) NSCLC, effectively defining the relevance of a disease subtype in lung cancer by its response to a targeted therapy. [4,5] Despite the early successes of crizotinib, it is clear that many challenges still lie ahead for those who treat, or are affected by, ALK+ NSCLC. This review will examine both what we already know and the major emerging questions associated with optimal management of this disease.

The biology of native ALK

ALK is located on chromosome 2 and encodes a transmembrane receptor tyrosine kinase in the insulin receptor superfamily, with homology to the leukocyte tyrosine kinase. [6,7] Expression of native ALK in adult human tissues appears restricted to the small intestine, testis and nervous system. [2] In mice, expression in aspects of the embryonic and neonatal central and peripheral nervous systems suggests a role in neurological development. [6-8] In Drosophila, ALK has a proven role in the development of both the visual system and in visceral muscle patterning. [9-13] As with many other transmembrane tyrosine kinases, dimerization is thought to mediate the normal activation of the receptor. [14]

Activating ALK in NSCLC

Although primary activating mutations in ALK do occur, in most ALK+ cancers including NSCLC activation of ALK is through the formation of fusion genes (Box 1, Figure 1). [15-20] Multiple 5′ fusion partners for *ALK* occur in different ALK+ cancers. [20] In NSCLC, the dominant 5′ fusion partner is *EML4*, but other rarer partners, notably *KIF5B* and *TFG* have been described. [3,21,22] All 5′ fusion partners in oncogenic *ALK* rearrangements share certain key characteristics (Box 1). The 5′ partner and its associated promoter are known to influence both the expression levels and sometimes the sub-cellular location of the fusion protein. [23,24] Whether such variability introduces significant biological differences in terms of the downstream consequences of ALK activation is currently unclear.

The natural history of ALK positive NSCLC

ALK rearrangements in NSCLC are more common among those with adenocarcinoma histology, in never smokers and in those who are known to be wildtype for *EGFR* and *KRAS* (Box 2). [25] However, multiple ALK+ NSCLC cases that do not fit this clinical stereotype also exist. [26,27] Several different histological patterns such as signet-ring histology have been reported in association with ALK positivity but these are not specific. [28-30]

Given the lack of association with smoking, tobacco-related carcinogens are unlikely to underlie the etiology of most ALK+ lung cancers. While a number of general risk factors for non-smoking related lung cancer have previously been identified, including radon gas, no molecularly categorized epidemiological studies have been undertaken to explore the role of such factors specifically in ALK+ disease. [31]

The impact of ALK positivity on prognosis has been explored in resection series, where both better and worse survival outcomes compared to ALK negative groups have been reported. [32-35] In the advanced disease setting, if anything ALK positivity appears to be

associated with a neutral or slightly better prognosis than *EGFR/ALK* wildtype control groups. [36-38] However, although crizotinib naïve, high proportions of patients received pemetrexed within these studies (52-67%), a cytotoxic that has since been recognized to be particularly active in ALK+ NSCLC (see below). Consequently, we may never know the true prognostic value of ALK positivity in the absence of *any* key treatment in this setting.

The time to progression of advanced ALK+ NSCLC with most platinum-based first-line combination therapy appears similar to both *EGFR* mutant and *EGFR/ALK* wildtype patients. [29] Time to progression and response to erlotinib are comparable to *EGFR/ALK* wildtype patients but significantly lower than in *EGFR* mutant patients. [29] In contrast, pemetrexed, a multi-targeted anti-folate chemotherapy, appears to show exaggerated activity in ALK+ NSCLC. Among NSCLC patients treated with pemetrexed in different lines of therapy and in both mono- and combination therapy regimens, the median progression free survival (PFS) in ALK+ patients was 9 months (range: 1.5-21 months), compared to 4 months (range: 1-12.5 months) in an *EGFR/ALK/KRAS* wildtype control group. [39] Within a multivariate analysis adjusting for line of therapy, mono- vs. platinum and non-platinum combination therapy, age, sex, histology and smoking status, only ALK positivity was associated with a lower risk of progression on pemetrexed (HR = 0.36 (95% CI: 0.17-0.73, p=0.0051). [39] In addition, ALK positivity appears to be associated with a significantly higher response rate to pemetrexed (46.7%) than that seen in EGFR mutant or a double negative control group (4.7 and 16.7%, respectively; p=0.001). [40]

Two initial hypotheses have been raised to explain ALK+ NSCLC's apparent 'super-sensitivity' to pemetrexed. Firstly, that ALK positivity is associated with lower levels of thymidylate synthase, one of the targets of pemetrexed. [40] Secondly, that ATIC (5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/inosine monophosphate cyclohydrolase), a bifunctional enzyme that catalyzes the last two steps of purine biosynthesis may be both a substrate for ALK-mediated phosphorylation, increasing the cells dependence upon this pathway, and a direct target for pemetrexed. [39] Whether this apparent activity will affect the outcomes of the ongoing confirmatory randomized studies in advanced ALK+ NSCLC comparing crizotinib to either pemetrexed or docetaxel in the second line setting (PROFILE 1007; www.clinicaltrials.gov identifier: NCT00932893), or to a platinum-pemetrexed doublet in the first line setting (PROFILE 1014; www.clinicaltrials.gov identifier: NCT01154140) remains to be seen. Additional studies will also be required to determine if pemetrexed should now be prioritized as a cytotoxic in metastatic ALK+ NSCLC in the absence of access to crizotinib, after failure of crizotinib, and/or in the locally advanced, adjuvant or neoadjuvant setting, either with or without concurrent radiation therapy.

ALK positivity seems to be associated with a particular pattern of metastatic spread. At the time of diagnosis of metastatic disease, ALK+ patients, but not those with *EGFR* or *KRAS* mutations were associated with a higher incidence of pericardial (Odds ratio 4.61, p=0.02) and pleural spread (Odds ratio 4.8, p<0.001) than a triple negative control group. [41] The incidence of hepatic disease in ALK+ and *EGFR* mutant patients was also significantly higher than in the control group (Odds ratio 5.5, p=0.003). [41]

Early successes: Crizotinib's activity in single arm studies

Crizotinib (PF-02341066, Xalkori®) is a small molecule, orally bioavailable tyrosine kinase inhibitor (TKI) of both ALK and MET. [42] Within the first-in-man study of crizotinib, a series of molecularly defined expanded cohorts were recruited at the recommended phase II dose of 250mg BID in patients whose tumors were prescreened for evidence of either ALK or MET activation. [4] In addition to its activity in ALK positive NSCLC, clinical benefit of crizotinib has also been documented in both ALK positive lymphomas and sarcomas and in MET positive NSCLC and esophageal carcinomas. [90-93] ALK positivity within all of the initial studies was determined using break-apart FISH technology (Box 2). In the most recent NSCLC dataset from the phase I study, covering 119 patients of whom 116 were assessable for response, the objective response rate was 61%. [43] The high response rate appeared largely independent of age, sex, performance status or line of therapy. Median PFS was 10 months and although median overall survival data are not yet mature, the estimated overall survival rates at 6 and 12 months were 90% and 81%, respectively. [43]

Very similar results were obtained from the 136 ALK positive patients treated within the subsequent PROFILE 1005 single arm phase II study. [5] Within this study there was also evidence from patient reported outcomes of significant and sustained symptomatic benefit in pain, dyspnea, cough and fatigue after 6 weeks of therapy. [5] Although crizotinib received accelerated approval in the US in August 2011, this approval is conditional upon the results of ongoing randomized studies comparing crizotinib to standard chemotherapies (PROFILE 1014 and 1007). In both of these studies, patients randomized to the chemotherapy arm will have the option of crossing over to crizotinib upon progression. Consequently, the primary endpoint for both trials is PFS and not overall survival (OS). A prospective study that could formally address the effect of crizotinib on OS in advanced ALK+ NSCLC is unlikely to occur. However, when crizotinib treated patients were retrospectively compared with matched crizotinib naïve controls, the one and two-year survival rates significantly favored the crizotinib treated group (70% vs. 44% and 55% vs. 12%, respectively, p=0.004). [36]

Early successes: Crizotinib's tolerability in single arm studies

Although up to 96% of ALK positive NSCLC patients in the early studies of crizotinib reported treatment-related adverse events, the majority of these were only grade 1 or 2 in severity (Table 1). [4,5,43] Given the role of ALK in the development of the visual system and gut, it is tempting to speculate that several of the common side effects reflect direct anti-ALK effects on the native protein. Peripheral edema may be a notable exception, having also been described in association with MET inhibition. [44] The visual disturbances associated with crizotinib usually commence within days of starting the drug and involve brief light trails, flashes or image persistence occurring at the edges of the visual field. Most commonly, these occur in association with changes in lighting. Studies in rats have demonstrated that crizotinib causes significant reductions in the rate of retinal dark adaptation, but not the ability to achieve full dark adaptation, offering a partial explanation for these clinical findings. [45] Severe side effects are rare (Table 1). [4,5,43] Drug holidays, followed by rechallenge at a lower dose have been reported to allow ongoing treatment in

some cases of severe neutropenia or transaminitis, but permanent drug discontinuation is occasionally required. [4,43]

Rapid onset hypogonadism in the majority of male patients taking crizotinib has now been reported, suggesting that serum testosterone levels should be routinely checked and replaced as appropriate on therapy. [46] Cases of crizotinib-induced asymptomatic profound bradycardia have also recently been described, the clinical significance of which remains uncertain. [47]

Coming challenges: The need for newer ALK directed therapies - pharmacology, tolerability and resistance

Given the early successes, in terms of both activity and tolerability, of crizotinib in ALK+ NSCLC, it may seem strange to even consider the need for newer ALK directed therapies in this disease. However, several opportunities present themselves as suitable for improvement. For example, despite crizotinib having a half-life of over 50 hours, it is dosed twice a day primarily to abrogate Cmax related side-effects within the phase I study. [4, 83] Consequently, re-exploring crizotinib or exploring another ALK inhibitor as a once a day regimen may be attractive to many patients. Although crizotinib appears to be relatively well tolerated, it is not yet clear which of crizotinib's side-effects reflect activity against native ALK as opposed to other molecular targets (Table 1). Therefore newer ALK inhibitors with narrower, or at least different, spectra of activity, may offer different and in some cases more desirable side-effect profiles. However, the dominant reason to consider newer ALK-directed therapies is to address the challenge of intrinsic and acquired resistance to crizotinib in ALK+ NSCLC.

Coming challenges: Resistance to crizotinib in the central nervous system (CNS)

Despite the dramatic activity of crizotinib in ALK+ NSCLC, the disease will eventually progress in all cases. While on many occasions resistance to crizotinib will be manifested as systemic progression, in some situations CNS only progression occurs. [43] Accurate details on the exact proportion of times this occurs are lacking as standardized baseline and surveillance scans of the CNS were not part of any of the crizotinib trials conducted to date. Whether CNS progression represents the chance location of a change in the dominant biology of ALK+ NSCLC and/or unaffected growth secondary to relative under-exposure to the drug in the CNS is uncertain. Data do exist to support a pharmacokinetic explanation in some cases. In one patient with CNS progression, CSF drug levels were noted to be <0.3% of those seen in the blood, levels predicted to be too low to be effective against any ALK fusion proteins present. [48]

Consequently, further exploration of the potential for CNS progression to reflect a condition when local CNS treatment, e.g. radiation therapy, should be combined with ongoing use of the crizotinib to continue to suppress systemic disease is warranted. In addition, the potential for intrathecal delivery of crizotinib and formal assessment of meaningful CNS exposure with newer ALK-directed therapies should be considered as a means of assessing this potentially distinct mechanism of 'acquired' resistance to crizotinib.

Coming challenges: Resistance to crizotinib systemically

Biological drug resistance, whether acquired or intrinsic, poses a significant challenge to oncogene-targeted therapy. Based on the previous paradigm of *EGFR* mutant NSCLC, kinase domain mutations were expected to provide a mechanism of resistance to crizotinib in ALK+ NSCLC. In fact, the first published report of crizotinib's clinical activity was accompanied by a case report of acquired resistance secondary to two new kinase domain mutations, L1196M and C1156Y, each occurring in different clones from the same patient. [4,49] The L1196M substitution occurs at the gatekeeper position, homologous to the T790M in *EGFR* or the T315I substitutions in *BCR-ABL*. [50,51] Five additional *ALK* kinase domain mutations (L1152R, G1269A, S1206Y, G1202R and 1151Tins) have since been reported from NSCLC patients with crizotinib resistance, with several others, including D1203N and F1174C, already identified (Doebele, unpublished data). [52,53,94] Multiple other mutations that confer crizotinib resistance can be generated through *in vitro* mutagenesis screens on *EML4-ALK* positive cell lines. [54,55] Of note, one of these, F1174L, has now been identified clinically in a patient with ALK+ inflammatory myofibroblastic tumor (IMT), harboring a *RANBP2-ALK* translocation who developed crizotinib resistance. [56] F1174L is also a known activating mutation of full length *ALK* in neuroblastoma. [17,18] Increases in our understanding of crizotinib resistance mechanisms in ALK+ disease are therefore likely to be important across histologically diverse tumors.

Collectively, the available clinical and preclinical data suggest multiple different kinase mutations occurring at comparable frequencies may generate crizotinib resistance. This stands in contrast to the EGFR paradigm where T790M predominates and other EGFR TKI resistance mutations occur only rarely. [57,58,59] Crizotinib resistance mutations appear more analogous to the broad spectrum of mutations observed in *BCR-ABL* following treatment with imatinib. [60] Potentially, the ability of the EGFR to tolerate additional mutations in its kinase domain may be constrained by the presence of the common activating mutations. [61] As EML4-ALK, like BCR-ABL, is not activated via mutations, the kinase domain may be less constrained and able to tolerate multiple different new mutations that impair crizotinib binding without significantly impacting the kinase's underlying activity (Figure 2).

In support of this, several groups have demonstrated that ALK gene rearrangements with resistance mutations enhance the growth of cells compared to unmutated *ALK* rearrangements. [53,56] In contrast, a secondary T790M mutation reduces the growth of *EGFR* mutant cells. [62] This could have important clinical implications for ALK+ patients. Whereas the less 'fit' T790M clones may be competed out by re-emerging sensitive subclones in the absence of an EGFR TKI, crizotinib resistant ALK mutated clones may not regress so easily in the absence of an ALK TKI. [63]

The different *ALK* mutations identified thus far show variability in their sensitivity to crizotinib and to other ALK inhibitors suggesting that a single next generation ALK inhibitor may not be able to effectively inhibit the entire spectrum of resistance mutations. [54,55,94] Complicating this strategy further is the possibility of intrapatient heterogeneity in terms of different resistance mutations. [49] With *BCR-ABL* up to a ten different resistance mutations have been detected in a single patient using mass spectrometry

following progression on imatinib and with comparable analyses similar complexity may emerge in ALK+ disease after crizotinib therapy (Figure 2). [64] With differences in CNS penetration of some newer ALK-directed drugs we may even find that achievable exposures will suppress some mutations, but allow others to grow in different parts of the body with the exact pattern varying depending on the drug involved. Copy number gain (CNG) of the *ALK* gene fusion, either alone or in combination with a kinase domain mutation, has also been observed both *in vitro* and in patients as an additional mechanism of crizotinib resistance. [53,65,94]

Approximately 13% of patients with an *ALK* gene fusion demonstrate the presence of an additional mutated oncogene, specifically *EGFR, KRAS, BRAF* or *MET*, in their pre-crizotinib sample. [66] Potentially, selection of these or other alternate means of oncogene activation could provide a mechanism of resistance to ALK inhibitors distinct from ALK kinase domain mutations and ALK CNG. Of note, MET is unlikely to function as a driver of resistance to crizotinib as it is a target of the same drug, however it could act as a driver of resistance for other non-MET directed ALK inhibitors. In DFC1032, a cell line derived from an *ALK*+, treatment naïve patient, both an *ALK* gene rearrangement and activation of EGFR and HER2 were noted. [67] In another cell line, derived from a patient with acquired resistance to crizotinib (DFC1076) both an L1152R resistance mutation in *ALK* and enhanced baseline EGFR and MET phosphorylation were noted. [52] No HER family CNG or mutations were detected and ligand-mediated activation of a second driver pathway was therefore proposed as contributing to crizotinib resistance in both cases. In both cell lines growth inhibition by the combination of crizotinib and an irreversible inhibitor of EGFR and HER2 was more effective than either treatment alone. More recently, 3 of 11 ALK+ patients with resistance to crizotinib were shown to have a detectable *EGFR* mutation or a *KRAS* mutation in their post-crizotinib specimen. [53] Unlike with cell line data, the presence of different oncogenes in the same/subsequent biopsies could represent either direct mechanisms of resistance occurring within the same cells (second drivers), or the emergence of independent/divergent clones with separate drivers (Figure 3). Certainly, introduction of a mutated *EGFR* cDNA can induce crizotinib resistance in an ALK+ cell line, consistent with the possibility of an *EGFR* mutation acting as a second driver. [52] In contrast, expression of a mutant *KRAS* cDNA into an ALK+ cell line does not. [53] An explanation for this finding may lie in previous work demonstrating that activated KRAS does not always behave as a classical driver oncogene and its role in oncogenesis may be highly contextual. [97] In addition, in a cell line derived from an ALK+ patient at the time of progression on crizotinib only a *KRAS* mutation was detectable without evidence of a persisting *ALK* gene rearrangement, suggesting that two separate clonal populations each with a separate driver co-existed in the same patient. Case reports of patients demonstrating both ALK positivity and an *EGFR* mutation who have responded to an EGFR TKI alone, rather than requiring a combination of drugs, also suggest separate drug sensitive clonal populations can co-exist in ALK+ patients. [68] Recently, amplification of the *KIT* gene has been reported as an additional alternate oncogene that may drive resistance to crizotinib in selected cases. [94] *KIT* amplification (defined as a ratio of the *KIT* gene to centromere 4 of >5) was noted in 2 of 18 cases of acquired resistance to crizotinib (1 focally and 1 diffusely within the tumor) with

accompanying preclinical evidence of the potential for KIT CNG to drive crizotinib resistance as a second driver in the presence of its cognate ligand stem cell factor (SCF). [94]

From the resistance data collected this far it appears that systemic resistance to crizotinib in ALK+ NSCLC can be parsed into two groups, those that are still dominated by ALK signaling (kinase mutation and CNG) and those that are only partially dependent on, or independent of, ALK signaling (presence of a second or separate oncogene driver) (Figure 3). Based on one of the largest series to date, the sizes of these two groups may be approximately equal. [53] However, given that clinical and preclinical examples positive for both kinase domain mutations and evidence of second or separate drivers have now been described, it is important to recognize that multiple different resistance mechanisms may be generated and co-exist at different levels in the same crizotinib-resistant patient. [52,94] Next generation ALK inhibitors alone, unless they also target other relevant kinases, may overcome some resistance mutations or CNG through higher exposures and/or higher potency, but are unlikely to overcome resistance acquired through second or separate drivers. [53,54] In such cases, combination therapy may be required. [52,67] However, while the technology for detecting mutations or CNG is well established, identifying those who require specific combinations based on increased signaling of an alternate oncogene alone, as in the DFC1032/1076 cell line data, is not yet feasible for routine clinical samples. For example, in a recent study looking at phospho-EGFR levels by IHC in paired pre- and post-acquired resistance to crizotinib samples, although the study reported 4 of 9 cases with subjectively increased staining as evidence of EGFR pathway activation as a potential mechanism of resistance, levels remained the same in 3 cases and went down in two cases. [94] Although intriguing and consistent with preclinical evidence, several factors including standardization of sample fixation, preparation and IHC quantitation might influence changes in phoshpo-EGFR levels given the well-known lability of phospho-epitopes. [94] Two other pharmacological classes of agent are being actively explored for their activity in crizotinib-resistant ALK+ NSCLC: pemetrexed and HSP90 inhibitors. However, just as with the new ALK inhibitors, recognizing that different biological mechanisms of crizotinib resistance exist is likely to have major impact on the development of these agents in this setting.

Currently, the activity of pemetrexed has been demonstrated in ALK+ crizotinib naïve patients, but not in crizotinib resistant patients. [39,40] It is also not yet clear whether ALK positivity is simply associated with pemetrexed sensitivity, or whether ALK signaling per se drives that sensitivity. If it is the latter, then mechanisms of resistance that preserve aspects of ALK signaling might retain marked sensitivity to pemetrexed, while if resistance is through the emergence of ALK negative clones pemetrexed may be less effective.

An alternate strategy for overcoming ALK resistance is to target the chaperone pathway. HSP90 is a chaperonin that stabilizes proteins during their maturation within the cell. [69] Multiple different oncogenic proteins have been described as potential clients for HSP90, however not all of them are equally HSP90 dependent. ALK fusion proteins were first described as highly sensitive to HSP90 inhibition in preclinical models of NPM-ALK. [70] Subsequently, EML4-ALK was also shown to be highly sensitive to HSP90 inhibition both in vitro and in vivo in crizotinib naïve patients. [71-73] Unfortunately, with regard to their

activity in crizotinib resistant disease, two such patients have already been noted to progress through HSP90 inhibitor therapy without evidence of benefit. [73] While HSP90 inhibitors have been shown to have activity across *EML4-ALK*+ cell lines harboring a range of different crizotinib resistance mutations, suggesting their clinical spectrum of activity may be broader than some specific ALK inhibitors in this context, notably the mechanism of crizotinib resistance had not been explored in either of the two patients described. [65,94] Similar to pemetrexed, it is likely that different mechanisms of resistance to crizotinib will result in retained or reduced sensitivity to HSP90 inhibitors compared to a crizotinib-naïve ALK+ population.

Whether effective levels of pemetrexed or HSP90 inhibitors will penetrate the CNS with regard to ALK+ brain metastases is unknown. Similarly, what biological mechanisms and how easily they will occur as a means of generating acquired resistance to these different classes of drugs in the post-crizotinib setting is also currently unknown. [74]

Coming challenges: Ascribing value to local therapy for ‘oligo-progressive’ disease plus treatment with crizotinib beyond progression

Even in situations where the mechanism of resistance is unknown or without an obvious drug-based therapy to direct towards it, resistant disease may sometimes be amenable to local therapy, such as stereotactic body radiation therapy. The logic behind this approach is that if resistance arises clonally, in conjunction with close CT or PET/CT observation, isolated areas of growth may be identified and deleted prior to more widespread dissemination of the resistant disease. [75-77] Within the Phase I study of crizotinib, thirty-seven patients continued to receive crizotinib for >2 weeks post-progression at the investigator’s discretion, 11 for more than 6 months from the time of their initial protocol-defined progression. [43] Although no formal details are available, in many cases this continuation likely occurred after areas of localized progression in both the body and/or CNS were treated with radiotherapy. How we formalize the definition of ‘ongoing clinical benefit’ to determine the true utility of attempted clonal deletion and continuing crizotinib in this setting will be yet another challenge in the future for determining the optimal management of ALK+ disease.

Conclusions

ALK+ disease has emerged as a relevant clinical subtype of NSCLC based on its dramatic and prolonged benefit from crizotinib. With increased experience, key features associated with the disease are now being revealed (Box 2). The greatest challenges associated with optimally treating this disease in the future will include identifying and managing the side effects associated with therapy (Table 1), determining which of these side effects are likely to be class specific in the face of new ALK directed treatments being developed and addressing the multiple different ways in which ALK+ disease may become resistance to crizotinib (Figure 3).

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Box 1: Activation of ALK as an oncogene in NSCLC and other cancers

Native ALK is a tyrosine kinase primarily involved in developmental processes. It is only expressed at low levels in adults, in the gut, testis and nervous system, where it's normal function remains under investigation. [20] ALK activation has been described in a number of different cancers including subsets of non-Hodgkins lymphoma, inflammatory myofibroblastic tumors, rhabdomyosarcomas, NSCLC, renal cancer, anaplastic thyroid cancers and neuroblastomas. [15-20,95,96] In cancers arising from tissues in in which full-length ALK is normally expressed, or, conceivably, where it becomes re-expressed during malignant dedifferentiation oncogenic activation may involve point mutations in the ALK kinase domain (e.g. neuroblastoma and anaplastic thyroid cancer). [15-19] In most tumors, however, including NSCLC, in order for ALK to function as an oncogenic driver, gene fusions that can induce both *de novo* ALK expression *and* activation are required. Multiple different 5′ fusion partners for oncogenic ALK exist in different cancers that all share the following characteristics: a) a promoter region that is functional in the tissue of interest and b) an encoded region capable of mediating oligomerization of the fusion protein, activating the ALK kinase domain in a manner comparable to the ligand-mediated oligomerization of native ALK. [20] In addition, there are presumably key structural constraints that must be met in both the 5′ partner and the specific breakpoint selected in the gene for the resulting fusion protein to be stable. While tissue specific expression of the 5′ partner could influence the 'preferred fusion partnerings' seen in some ALK+ malignancies, in many cases the 5′ partners represent very widely expressed proteins. Therefore, why EML4 is the dominant fusion partner in NSCLC, and not, for example, clathrin heavy chain (CLTC, a ubiquitously expressed endocytic scaffold protein) or any of several other very commonly expressed genes that have been reported as 5′ partners in other ALK+ malignancies, remains unknown. [78,79]

Box 2: Features associated with ALK+ NSCLC*

- Median age of onset in 5th or 6th decade, but with a broad age range extending from 2nd to 8th decade in adults [26]
- Adenocarcinoma histology (especially with signet ring histology) [26]
- Never/light smoking status [26]
- Minimal overlap with other oncogenic driver mutations [26,66]
- Excess of hepatic metastases, pleural and pericardial effusions at presentation with advanced disease [41]
- Prolonged progression free survival and high response rate to pemetrexed [39,40]
- ALK rearrangements may originate in a ‘near-diploid’ state, before significant chromosomal aneusomy occurs [84]

*Exceptions to all of the listed clinical features occur

Box 3: Finding ALK positive patients

ALK rearrangements are found in approximately 3-7% of NSCLC, in series dominated by adenocarcinoma histology. [26] Within the initial crizotinib studies, all patients were proven to be ALK+ using the Vysis break apart FISH probe set and it was this technology that was filed as a companion diagnostic with the FDA. [4,5] In break-apart testing, the FISH probes flank the common breakpoint in *ALK* and separate when a rearrangement occurs. [25] However, several other techniques, notably immunohistochemistry (IHC) and reverse transcriptase polymerase chain reaction (RT-PCR), are being developed. Each has both pros and cons. [80,81] Whether physicians or payers in the USA and in other countries will restrict prescribing only to those proven to be ALK+ using a specific methodology, or whether prescribing in cases proven to be ALK+ by any technique will occur remains to be seen. In addition, as ALK positivity only occurs in a small proportion of NSCLC and testing resources may be limited, various screening strategies to determine who to test have also been proposed. [26] These range from clinical enrichment using the factors associated with ALK positivity listed in Box 2, to tiered molecular testing. [25, 82] In tiered testing, the commonest molecular abnormality in NSCLC is assessed first and then, if negative, the next most common marker is assessed, etc until a positive result is achieved. Much of the basis for adopting or not adopting any given technique will be its sensitivity and specificity, its reproducibility over time and between centers and its cost when optimized for performance and reproducibility. Much of the basis for adopting any given screening strategy will be in balancing a) the cost savings in terms of reducing the absolute numbers of patients screened and the cost per positive within an enriched population, and b) the number of true positives missed by any pre-selection approach. [27]

Keypoints

- Crizotinib shows significant benefit in terms of both radiographic response and PFS in ALK+ NSCLC.
- Crizotinib is generally well tolerated, but is associated with characteristic common mild (e.g. gastrointestinal disturbance, visual changes and low testosterone) and rare serious side effects (e.g. transaminitis).
- Crizotinib-resistance in ALK+ NSCLC occurs through multiple different biological mechanisms including kinase domain mutations and copy number gain in the rearranged gene that preserve ALK dominance, and the emergence of ‘second’ or ‘separate’ oncogenic drivers which may weaken or negate ALK dominance, respectively.
- In addition to a change in the biology of the cancer, cases of isolated CNS progression on crizotinib could reflect inadequate CNS penetration of the drug.
- Newer ALK inhibitors, HSP90 inhibitors and pemetrexed have all shown preliminary clinical or preclinical activity in ALK+ NSCLC and are being investigated further in crizotinib resistant and crizotinib naive ALK+ disease.
- The potential of these new agents to have activity on CNS disease and on crizotinib resistant cases manifested through different biological mechanisms will be critical in determining their future roles in the management of ALK+ NSCLC.

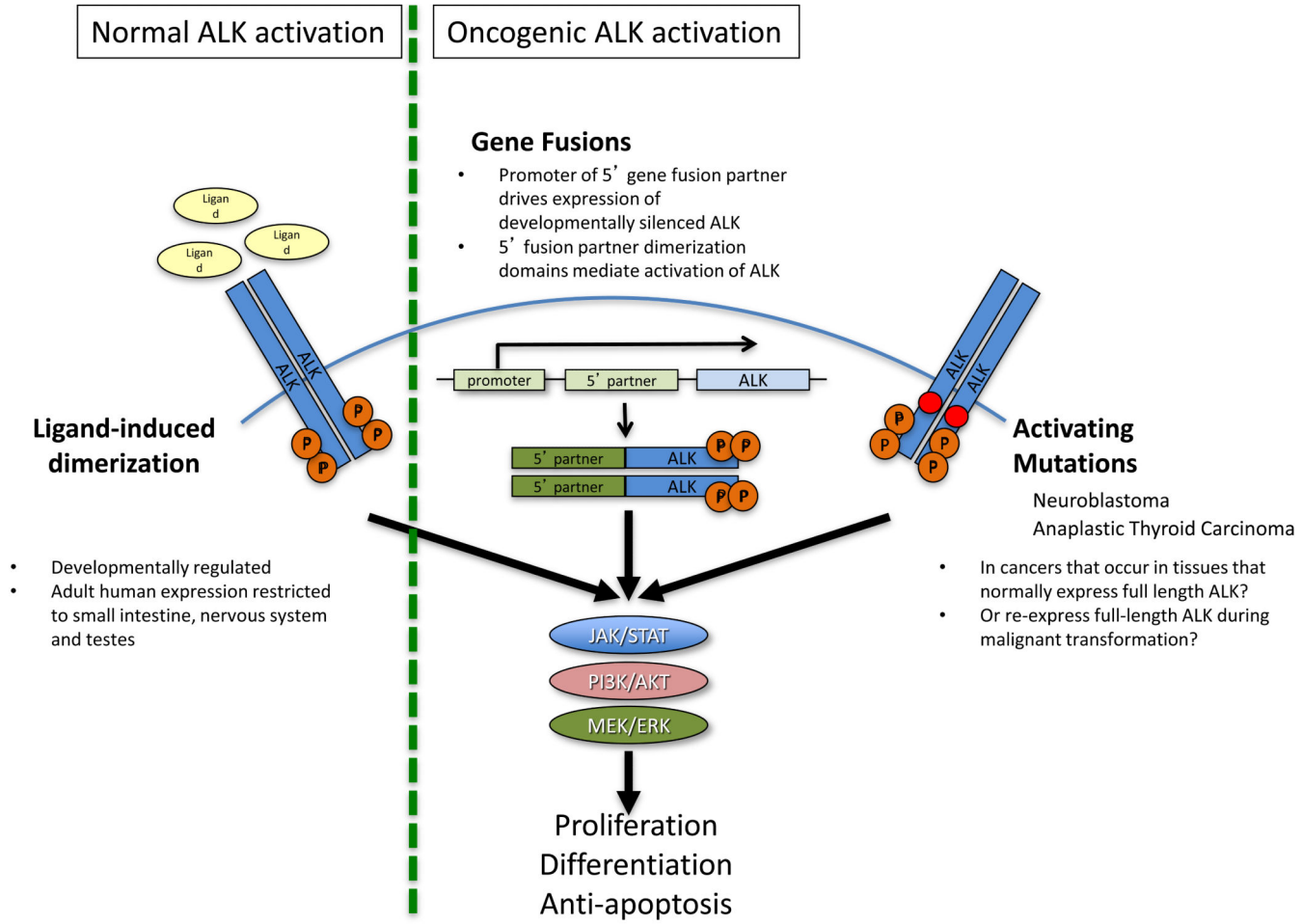

Figure 1. ALK activation mechanisms

Activation of ALK signaling is rare in most adult tissues but is active in the development of the gut and nervous system. Activation of native ALK is via ligand-induced dimerization and resultant autophosphorylation. In drosophila the ligand for ALK is Jelly Belly, whereas in mammals pleiotrophin and midkine have been reported as ligands for ALK. [11,12,86,87] In most ALK+ cancers, ALK expression is re-instituted through the active promoter of a 5′ partner that fuses with the kinase-encoding region of ALK. The resulting fusion gene then generates a fusion protein that can dimerize via domains in the 5′ partner mimicking ligand induced activation. [14] Rarely, mutations in the kinase domain of full length ALK can also promote primary oncogenic activation of ALK (Box 1). ALK phosphorylation results in activation of downstream signaling pathways including JAK/STAT, PI3K/AKT, and MEK/ERK, which can promote cell proliferation, differentiation, and provide anti-apoptotic signals. [88]

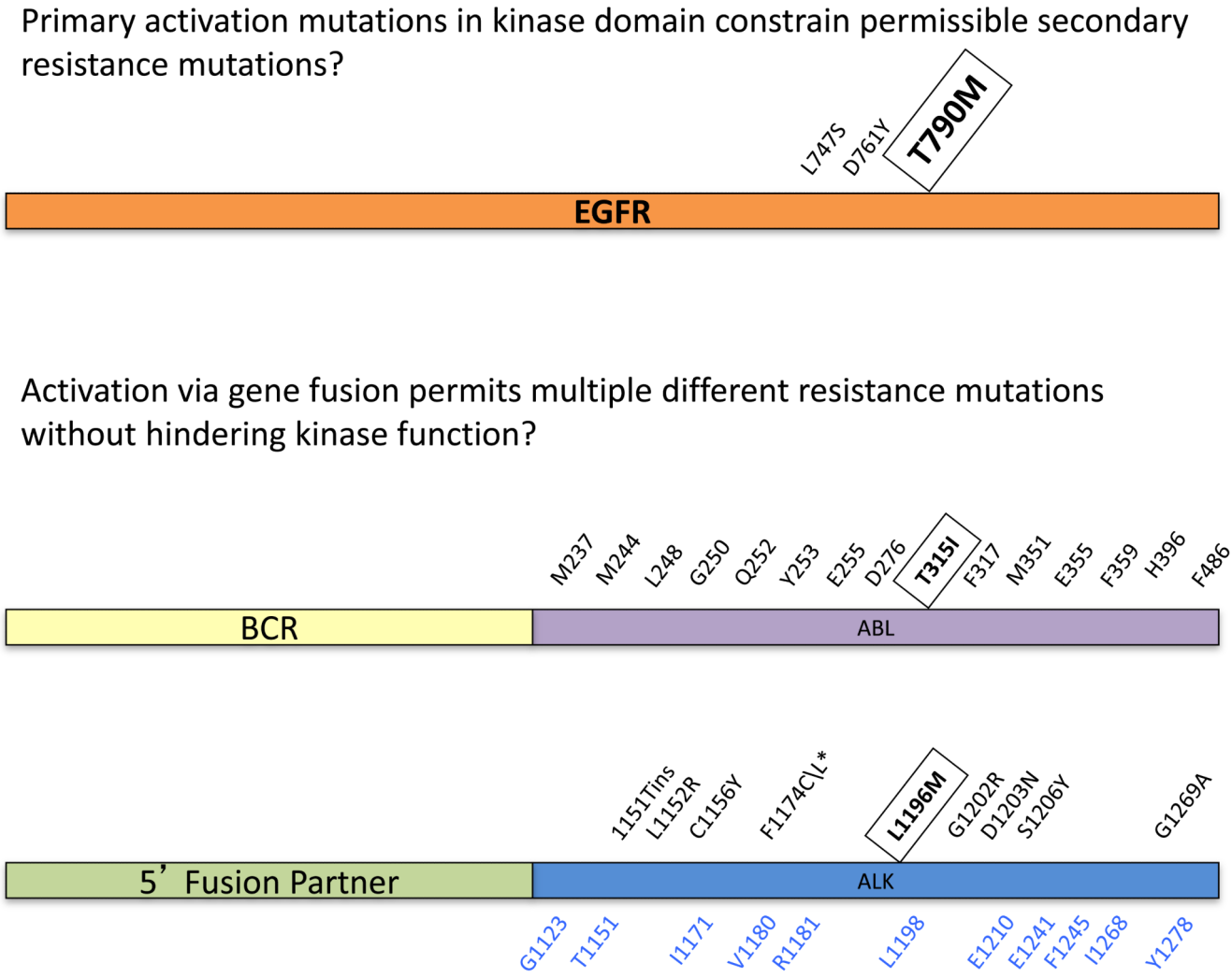

Figure 2. Resistance mutation spectra in EGFR mutant, ALK+ and BCR-ABL+ cancers following treatment with relevant kinase inhibitors

The spectra of mutations found in EGFR mutation positive NSCLC, ALK+ cancers, and BCR-ABL+ chronic myeloid leukemia are shown. We hypothesize that activating mutations such as those found in EGFR constrain the resistance mutations to those that allow constitutive activation. In situations where activation of the kinase is driven by dimerization via a gene fusion partner (e.g., BCR-ABL and EML4-ALK), the kinase domain is less constrained and can accommodate a wider array of mutations. The gatekeeper mutation, which sits at a critical position in the ATP/kinase inhibitor pocket, in each oncogene is enclosed with a box. The predominant resistance mutation in EGFR following therapy with erlotinib or gefitinib is T790M (shown in larger font) with only rare reports of other resistance mutations occurring. [57-59] The most common resistance mutations in BCR-ABL are shown. [89] T315I, the most frequent of these, only occurs <15% of the time. The true prevalence of the different ALK mutations that produce resistance to crizotinib in patients is still unknown but a single dominant form comparable to the status of T790M does not seem apparent among the patients studied to date. [49,53,56,94] Almost all of the resistance mutations

found in patients have also been identified using in vitro screens. [54,55] Additional resistant mutations identified through these screens but not yet identified in patients are shown in blue. The asterisk denotes a mutation, F1174L, that was found in an IMT tumor with the RANB2-ALK gene fusion treated with crizotinib; all other mutations were found in ALK+ NSCLC patients. [56]

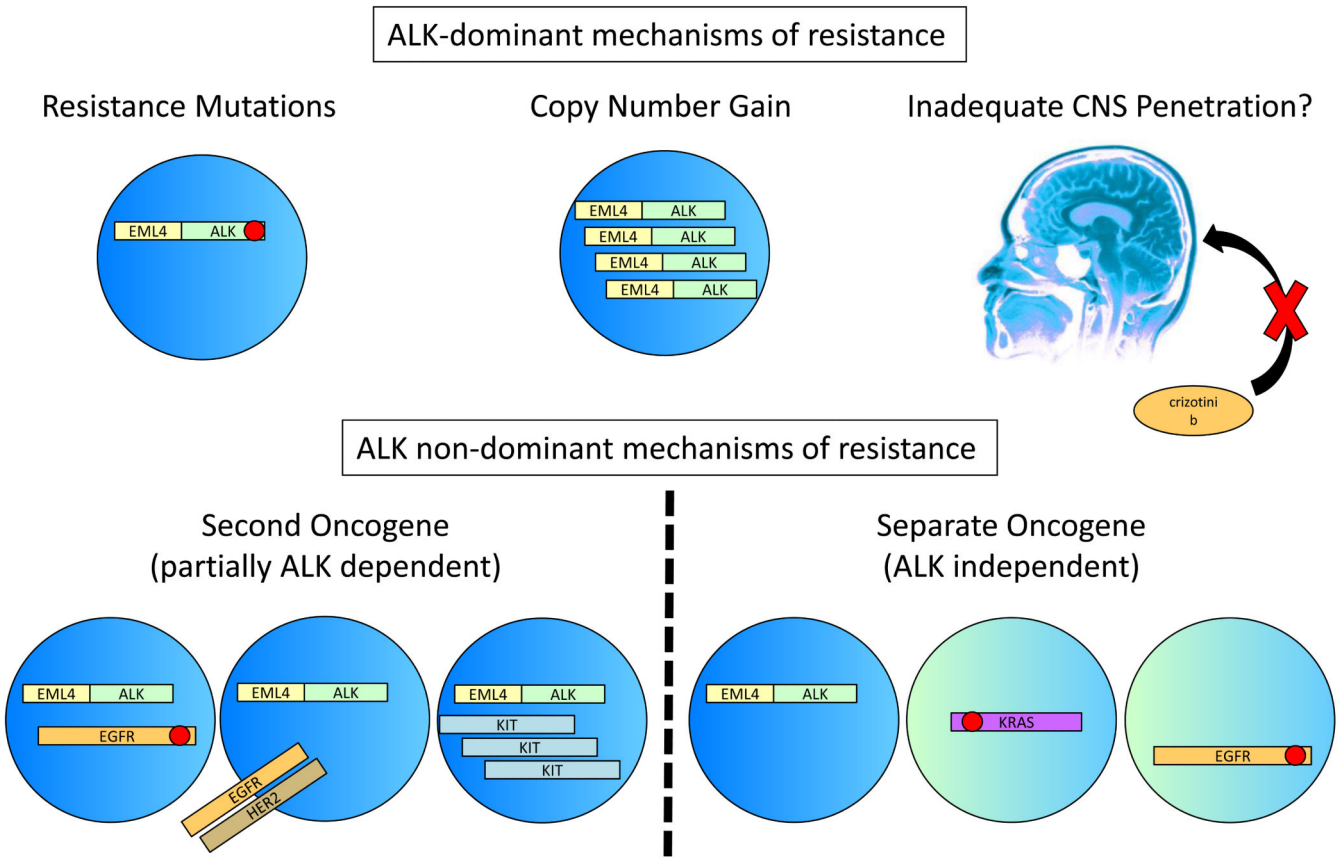

Figure 3. Mechanisms of resistance to crizotinib in ALK+ NSCLC

Crizotinib resistance can be classified into two broad categories, those mechanisms that retain the dominance of ALK signaling and those that lose the dominance of ALK signaling either partially or completely. ALK-dominant resistance can occur through kinase domain mutations that inhibit crizotinib binding but permit ongoing constitutive activation of ALK. [49,53,56] ALK dominant resistance also occurs through copy number gain of the ALK gene fusion, which may co-exist with resistance mutations. [53, 65] Finally, poor penetration of crizotinib into the CNS may simply allow unaltered ALK+ cancer cells to grow because of inadequate local drug exposures. [48] For resistance that degrades the dominance of ALK signaling, a second oncogene may become active via mutation or another mechanism co-existing with oncogenic ALK in the same cells. [52,53,65,94] Alternatively, true ALK independent resistance may arise through the outgrowth of clones that do not harbor an ALK gene fusion and contain a different activated oncogene (separate oncogene). [53]

Table 1

Common, severe and/or characteristic side-effects attributed to crizotinib [4,5,43,46,47,85]

Common treatment related adverse events (all grades) occurring in ≥10% of patients	All Grades
Vision Disorder (usually involving brief light trails, flashes or image persistence occurring at the edges of the visual field. Most commonly, these occur in association with light adaptation)	62%
Gastrointestinal Disorders	
Nausea	53%
Diarrhea	43%
Vomiting	40%
Constipation	27%
Decreased Appetite	19%
Esophageal Disorders	11%
General Disorders	
Edema	28%
Fatigue	20%
Other	
Dizziness	16%
Neuropathy	13%
Dysgeusia	12%
Rash	10%
Investigations	
Alanine Aminotransferase Increased	13%
Severe (grade 3 or 4) treatment related adverse events (all <1%-5% of cases)	
Stomatitis	
Constipation	
Fatigue	
Dyspnea	
Neuropathy	
Pneumonitis	
Aspartate Aminotransferase Increased	
Alanine Aminotransferase Increased	
Neutropenia	
Lymphopenia	
Hypophosphatemia	
Additional recently described common and/or characteristic side effects	
Renal cysts (rare)	
Asymptomatic bradycardia (frequency unknown)	
Rapid onset low testosterone in men (common)	