Title

Treatment of *ALK* rearranged non-small cell lung cancer - a review of the landscape and approach to emerging patterns of treatment resistance in the Australian context

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Abstract:

Since the identification *anaplastic lymphoma kinase* (*ALK)* gene rearrangements in non-small cell lung cancer in 2005, the treatment of *ALK*- rearranged non-small cell lung cancer (*ALK*+ NSCLC) has evolved at a rapid pace. This molecularly distinct subset of NSCLC has uniquely important biology, clinicopathologic features and mechanisms of drug resistance which impact on the choice of treatment for a patient with this disease.

There are multiple ALK tyrosine kinase inhibitors (ALKi) now available in clinical practice with efficacy data continuing to emerge and guide the optimal treatment algorithm.

A detailed search of medical databases and clinical trial registries was conducted to capture all relevant articles on this topic enabling an updated detailed overview of the landscape of management of ALK-rearranged NSCLC.

Key Terms

ALK; non-small cell lung cancer; tyrosine kinase inhibitors; resistance; survival

Synonyms for ALK-rearranged NSCLC used in this report

ALK; ALK-positive; ALK+; ALK-rearranged; ALK-translocated; ALK-addicted

Abbreviations

ALK; ALK+- anaplastic lymphoma kinase rearranged non-small cell lung cancer; NSCLC- non-small cell lung cancer; ALKi- ALK inhibitor; EGFR- Epidermal growth factor receptor; KRAS- Kirsten rat sarcoma viral oncogene; TKI- Tyrosine kinase inhibitor; EML4- Echinoderm microtubule like-4; PFS- Progression free survival; ORR- Objective response rate; WHO- World Health Organization; FISH- Fluorescence in situ hybridisation; IHC- Immunohistochemistry; cDNA- Circulating free deoxyribonucleic acid; RT-PCR- Reverse-transcriptase polymerase chain reaction; KD- Kinase domain; MRI-B- magnetic resonance imaging of the brain; FFPE- Formalin fixed paraffin embedded; NGS- Next generation sequencing; ROS1- Proto-oncogene tyrosine-protein kinase; m- Median; RECIST- Response evaluation criteria in solid tumours; PBS- Pharmaceutical Benefit Scheme; TGA- Therapeutic Goods Association; GI- Gastrointestinal; TTP- Time to progression; RT- Radiotherapy; RP2D- Recommended phase II dosing; BD- Twice daily; ATP- Adenosine triphosphate; OS- Overall survival; NCI- National Cancer Institute; MATCH- Molecular analysis for therapy choice; CTC- Circulating tumour cell; FA-FISH - modified FISH assay with filtration enrichment

1. Introduction

Anaplastic lymphoma kinase (*ALK*) gene rearrangements are found in approximately 3-7% of both Caucasian and Asian patients with non-small cell lung cancer (NSCLC) and represent a distinct molecular subtype of lung cancer. (1) With an estimated 1.8 million new cases of NSCLC in the Western world in 2012, this translates into more than 90,000 patients diagnosed with ALK-positive(+) NSCLC per year. (2) In Australia the estimated incidence of lung cancer in 2017 is 12,434 cases translating to over 600 new cases of *ALK*+ NSCLC per year. (3)

First described by Soda and colleagues in 2005, *ALK* gene rearrangements are associated with distinct clinical and pathologic features, such as younger age, light or never smoking history. (1, 4, 5) Furthermore, with rare exceptions, the *ALK* rearrangement is typically found in adenocarcinoma, and tend to be mutually exclusive with other oncogenic drivers, such as *EGFR* and *KRAS* mutations. (4, 6, 7)

Chromosomal rearrangements of *ALK* result in a fusion oncogene in which the tyrosine kinase domain of ALK comes under the control of the promoter of a different gene (**Figure 1**) leading to ligand independent constitutive activation of the tyrosine kinase and its downstream signalling pathways (**Figure 2**). (8) The most common rearrangement is with *echinoderm microtubule-associated protein-like 4 (EML4)*-due to a chromosomal inversion within the short arm of Chromosome 2; (1) however, rearrangements can also occur with a number of different genes (**Figure 1**). (1, 9)

Identification of an *ALK* rearrangement is key as it confers sensitivity to treatment with ALK tyrosine kinase inhibitors (TKIs; ALKi). The use of these inhibitors have resulted in major clinical advances over the last decade, with superior progression free survival (PFS) and objective responses (ORR) over conventional chemotherapy in *ALK*-positive patients. However, acquired resistance to TKIs is inevitable in *ALK* patients who initially respond to treatment. This resistance has been associated with secondary somatic *ALK* tyrosine kinase domain mutations, *ALK* copy number gain, or the emergence of bypass pathway signalling. (10, 11) Identifying the various resistance pathways is critical to developing new treatment strategies and overcoming acquired resistance.

2. Objective

The landscape of treating *ALK* rearranged NSCLC is rapidly evolving. The objective of this review is to provide a summary of the current treatment approach to *ALK* rearranged NSCLC, highlighting the therapeutic challenges in the context of current literature evidence and the Australian context.

3. Methods

A comprehensive literature review was performed in Embase, Medline, Premedline and Cochrane databases, in addition to ClinicalTrials.gov and WHO. (12, 13) The terms ‘ALK’ or ‘anaplastic lymphoma kinase’ adjoining ‘positive’ or ‘rearranged’ or ‘re-arranged’ or

'translocated'; attached with 'carcinoma', 'non-small cell lung' or 'non-small cell' or 'adenocarcinoma' referenced anywhere within the title or article were grouped further with 'lung' or 'lung neoplasm' thus to capture all pertinent articles in the both pre-clinical and clinical setting. Results were not filtered for date or language.

At data search update (May 31, 2017): 668 articles were identified in Medline; 640 via Embase; 44 in Premedline and 15 Cochrane review articles. There was considerable article overlap inter-database. Articles relevant to the topic were selected manually via the search strategy results.

4. Discussion

4.1 Detection of *ALK*-rearranged NSCLC

ALK gene rearrangements or the resulting fusion proteins may be detected in tumour specimens using fluorescence in situ hybridization (FISH), immunohistochemistry (IHC), reverse transcription polymerase chain reaction (RT-PCR) and next generation sequencing (NGS). (14)

ALK break-apart FISH assays remain the gold standard due to their high sensitivity and specificity in identifying *ALK* rearrangements and it is mandated for clinical trial inclusion (**Figure 3**). (15, 16). High sensitivity ALK IHC with next generation antibody clones (such as 5A4 and D5F3) has performed more favourably than first anticipated, due to its relative accessibility and rapid-throughput method, high-sensitivity (up to 100%) and cost-effectiveness. (17) In the clinical setting, many centres in Australia now routinely screen all cases of non-squamous NSCLC for *ALK* gene rearrangement by IHC followed by confirmatory FISH testing only in ALK IHC cases. (18) Neither FISH nor IHC can identify the fusion partner. However, the exact clinical relevance of different fusion partners is not well characterised.

The identification of both the *ALK* rearrangement and a fusion partner is possible by RT-PCR and NGS technologies and RT-PCR was a commonly used screening strategy for detecting the most frequent *ALK* gene fusion partners. Assays have been designed for particular ALK fusions, which, while 100% specific for these fusions, are unable to detect novel fusion partners. Each newly identified fusion thus requires a new assay, making testing uneconomical and this testing strategy is no longer recommended. In addition, it can be difficult to obtain high quality RNA from routine formalin fixed paraffin embedded tissue and RT-PCR is not currently recommended by the CAP/IASLC/AMP guidelines to select patients for ALK-TKI treatment.(19)

The ability of targeted NGS to economically sequence large amounts of DNA and identify structural variations may see this testing introduced more routinely in the future for the identification of both known and novel *ALK* arrangements. (20)

4.2 Efficacy and Safety of *ALK* inhibitors

4.2.1 First generation ALK inhibitor- Crizotinib

Crizotinib is an oral small-molecule TKI targeting ALK, MET and ROS1 tyrosine kinases. (21) In two single-arm studies, crizotinib showed marked antitumor activity in patients with advanced ALK-positive NSCLC, with an ORR of approximately 60%. (22)

In 2013 the first randomised control phase III trial, PROFILE 1007 confirmed the benefit of crizotinib over conventional second line chemotherapy with a median (m)PFS of 7.7 months (mo) versus 3mo (**Table 1**). Symptom burden of cancer and quality of life was also improved.

In 2014, the first line phase III PROFILE 1014 trial affirmed the superiority of crizotinib over standard of care platinum doublet chemotherapy with a median PFS of 10.9mo over 7mo. (23) Cross-over was allowed and was high in this trial (70%) attenuating a likely overall survival (OS) benefit. Cancer specific symptoms and quality of life were again improved. One-quarter of patients on this trial had central nervous system (CNS) disease at diagnosis, and 50% progressed with CNS relapse. This study argued for crizotinib as the new standard of care over chemotherapy in this population (**Table 1**).

Crizotinib (Xalkori®) was initially approved by the US FDA in August 2011 and was available in Australia following listing on the Pharmaceutical Benefits Scheme (PBS) for treatment of patients in the first-line setting from July 1, 2015. All patients must provide evidence of *ALK* gene rearrangement in tumour material, defined as ≥ 15% positive cells by FISH with FISH testing reimbursed only for patients with ALK IHC+ tumours. (24)

Toxicity of Crizotinib

The two phase III crizotinib trials further established the unique side effect profile with crizotinib. This includes common side-effects of nausea, diarrhoea, peripheral oedema and transient visual disturbances, elevated transaminases and rare but serious hepatotoxicity and interstitial pneumonitis. (23, 25)

4.2.2 Second Generation *ALK* inhibitors

Endeavours to avoid or delay drug resistance have led to the development of second-generation agents with ceritinib, alectinib and brigatinib the most established. In February 2017 ceritinib (Zykadia®) became PBS reimbursed for use in *ALK* rearranged disease unrestricted by line of therapy, enabling two lines of treatment for patients (crizotinib then ceritinib). Alectinib (Alcensa®) was approved by the Therapeutics Goods Administration (TGA) in March 2017 for patients who have progressed on or are intolerant to crizotinib.

Second Line Treatment in Crizotinib Refractory Disease

Second-generation ALK inhibitors have generally been shown to have potent clinical activity, with actions against several crizotinib- resistant *ALK* Kinase Domain (KD) mutations and improved CNS penetration. (26) Second generation ALKi were first clinically evaluated in the

second line setting with substantially longer median PFS results than seen with chemotherapy (**Tables 1 and 2**). (27)

Ceritinib was the first second generation ALKi to show efficacy with reports of a 20-fold greater potency than crizotinib in enzymatic assays. (26) In the updated large phase I ASCEND-1 study in pre-treated patients ORR was 72% and mPFS 8.9mo whilst in the phase II ASCEND-2 trial PFS was 5.7 mo. CNS activity was superior to crizotinib. (27, 28)

In enzymatic assays, alectinib is approximately five times more potent than crizotinib. (29) In contrast to the other TKIs, alectinib does not inhibit the kinase activity of MET or ROS1. In the pivotal Phase II trial ORR in crizotinib refractory patients was 49% and mPFS 8.9mo. (30)

Early phase brigatinib data in a pre-treated population have also been promising. Preclinical activity has been demonstrated in patients with KD mutations conferring resistance to crizotinib, ceritinib and alectinib. (31) The updated ALTA trial reported a 15.6mo mPFS after crizotinib and an ORR of 62%. (32)

Entrectinib is a novel potent oral TKI with activity against patients with *ALK* in both the naïve and pre-treated setting also reported. (33) Phase I data have demonstrated safety and efficacy to carry this drug forward in trial as it has also demonstrated activity against a panel of resistance mutations. (34) Phase II trials with entrictinib has also commenced recruitment in a number of Australian centres (STARTRK-2) which will review the efficacy of this TKI in patients with *ALK* amongst other activating mutations, across multiple different tumour types. (12)

First Line Treatment with Second Generation ALK TKI

Ceritinib has not been compared directly with crizotinib in treatment naïve patients as at the commencement of the first line Phase III trial, platinum doublet chemotherapy was the standard of care. Patients treated with ceritinib had a mPFS of 16.1mo overall; 26.3mo in patients without brain metastases and 10.7mo in those with brain metastases at baseline. (35)

The Japanese J-ALEX trial confirmed impressive first line efficacy with alectinib compared with crizotinib with a PFS hazard ratio (HR) of 0.38 (99.7% CI: 0.26-0.55, stratified log-rank $p<0.0001$), mPFS was 25.9mo with alectinib versus 10.2mo with crizotinib and ORR 61.6% with alectinib. (36, 37) This trial was criticized for an unexpectedly high rate of discontinuation of crizotinib and was not stratified for CNS disease with more patients having brain metastases in the crizotinib arm. Subsequent data from the international Phase III ALEX trial confirmed superior efficacy with less toxicity of alectinib over crizotinib with overall mPFS in the alectinib arm not reached versus 11.1mo in the crizotinib arm (HR 0.47, 95% CI: 0.34-0.65; $p<0.0001$) and substantially less CNS progression (PFS HR 0.16, 95% CI: 0.10 - 0.28; P<0.001). These data establish a new standard of care for first-line treatment of ALK-rearranged NSCLC and will be used to support the application for alectinib reimbursement in Australia.

The first line brigatinib ALTA-1L trial versus crizotinib has now closed to recruitment and results are awaited (NCT02737501). Early phase data reported a confirmed ORR of 62% in crizotinib pre-treated patients and 100% in the crizotinib naïve population. (38)

The global phase III (eXalt3) is now recruiting, comparing entrectinib to crizotinib as first line therapy (NCT02767804).

Several differences between the studies exist including patient baseline demographics and disease status as well as inclusion criteria exist that do not allow for direct comparison (**Tables 1 and 2**).

Toxicity of Second Generation ALK Inhibitors

The second generation ALKi differ in their side effect profiles. Ceritinib is associated with diarrhoea in 85%, however ≥ grade 3 occurs in only 5%. (35) One suggested strategy is to introduce therapy at a lower than recommended dose and titrate up to mitigate gastrointestinal (GI) toxicity and raised transaminases. Alectinib is generally well tolerated with myalgias, Grade 1-2 GI toxicity and fatigue the most frequently reported toxicities. (30) Brigatinib has been found to induce an idiopathic elevation in creatine kinase (CK) and lipase and reports of an early, steroid responsive, pneumonitis. To alleviate this the phase III first line trial introduces brigatinib at a lower dose before dose escalation in the absence of symptoms. (38) Entrectinib is generally well tolerated, with low grade fatigue, dysgeusia, parasthaesias and nausea being most common. (34)

These toxicities may direct switch to an alternate ALKi if dose interruption, delay or pre-emptive/supportive treatment do not permit their ongoing use.

4.2.3 Third Generation *ALK* inhibitor- Lorlatinib

The safety and efficacy of lorlatinib has been established in the early phase setting in treatment naïve and pre-treated patients including those with CNS disease.

Preclinical data with lorlatinib revealed it is potent against ALK fusions and inhibits recognized KD mutations including the G1202R which infers resistance to prior ALKi. (39) The mOS in second line treatment was 13.5mo. Intracranial ORR was 39% with the CSF to serum ratio 0.61-0.96. Further durable efficacy has been reported in leptomeningeal disease. (40)

The spectrum of lorlatinib toxicity reported to date includes uncomplicated hypercholesterolaemia, hypertriglycerolaemia, peripheral oedema and reversible peripheral neuropathy. (40) A Phase III Study of lorlatinib versus crizotinib in first line treatment of patients with ALK+ NSCLC is now recruiting (NCT03052608).

4.3 Central Nervous System Disease in *ALK* NSCLC

Following the discovery of *ALK* rearrangements in NSCLC and the introduction of ALKi, it has become well recognised that CNS tropism and disease progression in the brain is a frequent complication in patients with *ALK*-positive NSCLC requiring vigilant monitoring and evaluation via baseline and sequential brain MRI. (25, 41) The CNS is proposed to be a

sanctuary site for patients with *ALK* rearranged NSCLC, with approximately 25% presenting with CNS disease, and up to 50% on crizotinib progressing in the CNS. (23, 41)

4.3.1 CNS disease in Crizotinib-treated patients

Crizotinib penetration into CSF is negligible (0.3% of plasmatic concentration). (42) Yet retrospective pooled analysis of the PROFILE 1005 and 1007 revealed median intra-cranial time to progression (TTP) in previously untreated CNS disease with crizotinib of 7mo and in those with prior treatment for brain metastases 13.2mo. (43) Prospective data in the PROFILE 1014 trial revealed crizotinib was superior overall to chemotherapy in patients with treated brain metastases (mPFS of 9mo versus 4mo; R 0.40; p<0.001). (41)

Prior targeted treatment of CNS with radiotherapy therefore improves the PFS on crizotinib; however, patients can still derive clinical benefit without pre-treatment. Better CNS penetration of systemic ALK targeted treatments are needed for patients with CNS disease from ALK+ NSCLC.

4.3.2 CNS disease in Second Generation ALKi patients

Newer generation ALKi have demonstrated superior CNS efficacy and given their improved penetration of the CNS, inclusion criteria for trials have not generally required pre-treatment with radiotherapy for asymptomatic CNS metastases.

Ceritinib has demonstrated CNS concentrations with a brain to blood exposure ratio in mouse models of 15%. (44) Disease control in the brain was achieved in 79% of patients with pre-treated CNS metastases. (27) Despite this, in the first line trial, 48% of patients with pre-existing brain metastases and 30% of those-without baseline CNS disease still progressed in the brain. Median PFS for those with baseline brain metastases was 10.7mo. First line ceritinib CNS ORR was 46.3%, with no significant difference in those pre-treated with radiotherapy (RT). (35)

In patients on second-line alectinib CNS ORR was 57%; 10/23 patients who had not had prior RT had a CNS complete response (CR). In the first line setting, 5 patients with CNS metastases at baseline treated with alectinib had CSF drug concentrations approximating unbound systemic concentrations. (45) The updated J-ALEX first line alectinib data found alectinib also prevented CNS progression compared to crizotinib (HR 0.51, 95% CI: 0.16-1.64) and delayed the onset of CNS disease (HR 0.19, 95% CI: 0.07-0.53). (37) In the ALEX trial intracranial ORR was 83%, mTTP again dramatically favoured alectinib over crizotinib (HR 0.16, 95% CI: 0.10-0.28; p<0.0001) and mPFS was not met in the alectinib arm with CNS disease (HR favouring alectinib over crizotinib 0.40, 95%CI: 0.25-0.64). (46)

The brigatinib CNS data reported to date are encouraging with ORR of 53% in patients not treated with prior RT and 56% in those who received previous CNS RT. (38)

The incidence of leptomeningeal carcinomatosis in *ALK*-rearranged NSCLC is unknown however it is expected to be higher than the prior reported incidence of 5% in NSCLC. (47). This is likely multi-factorial beyond CNS tropism, as imaging techniques have improved; with MRI of the brain now routine thus arguing for historical underreporting; therapeutic drug

concentration in CSF is difficult to achieve; and patients are living longer. (48) An intriguing case report found that dose escalation above the recommended phase II dose (RP2D) of alectinib to 900mg BD achieved an objective and durable response in a patient with leptomeningeal disease. (49)

Use of next generation ALKi can be seen as a radiotherapy sparing measure. Despite advances in treatment and survival, outcomes remain inferior to those in patients without CNS disease. Tumour genotyping of brain metastases may assist our further understanding of biological and molecular factors predicting for CNS relapse and progression.

4.4 Resistance to ALK Inhibitors

4.4.1 Resistance to Crizotinib

Unfortunately, some patients do not respond initially to crizotinib and acquired drug resistance inevitably develops in those who do respond. The mechanisms mediating such resistance have been described and our understanding continues to evolve with ongoing treatment advances (**Figure 4**). (10, 50)

ALK-Dependent Crizotinib Resistance

Different *ALK* fusion genes may have differing sensitivities to crizotinib with *EML4-ALK* variant 1 reported to have greater sensitivity than other *EML4-ALK* variants. (9) Beyond this, the development of *ALK* KD mutations can block the binding affinity of crizotinib as well as other TKIs and increase protein kinase activity. (51) These mutations are thought to account for approximately 25% of cases of crizotinib resistance. The gatekeeper L1196M mutation is the most common, characterised in approximately 30% of cases. (52)

Other mutations which confer variable levels of resistance have also been described, such as G1202R, G1269A and S1206Y. Some mutations such as C1156Y and L1152R confer resistance by allosteric hindrance of the binding site, decreased affinity for crizotinib, or interference with downstream signalling phosphorylation. As described in subsequent sections, second-generation ALKi have differing activities in the presence of the known KD mutations as the frequency of these mutations increases with each line of ALKi. (53-56)

Amplification / copy number gain (CNG) of the *ALK* fusion gene, alone or combined with secondary mutations, is responsible for the development of secondary resistance after crizotinib therapy in < 20% of cases. It is thought to allow for downstream signalling despite partial inhibition by crizotinib. (10)

ALK-Independent Crizotinib Resistance

Bypass signalling can also occur with the activation of, and constitutive signalling through, other pathways which have been increasingly described as resistance mechanisms to crizotinib. Activation of the EGFR pathway in this context usually occurs through increased

phosphorylation and upregulation of ligands. (57) Rarely, concomitant activating *EGFR* and *KRAS* mutations have also been described, possibly reflecting the emergence of pre-existing clones under selective pressure from crizotinib. (57, 58) Transformation to small cell lung cancer has been described in a patient who was treated with crizotinib and alectinib. (59)

Pharmacologic mechanisms such as inadequate penetration into the CNS, may also produce therapeutic resistance. (42)

Tissue and blood based biomarkers are being developed and validated to test for the heterogeneous and dynamic *ALK* dependent and independent mechanisms of resistance. Currently in 25% of cases the cause for resistance remains unknown. An attempt to obtain repeat tissue or liquid biopsy upon progression is recommended especially as NGS testing is becoming increasingly available and less expensive (**Figure 4**). (60-62)

4.4.2 Resistance Mechanisms to Next Generation Inhibitors

Apart from improved CNS penetration of next generation ALKi, alternative resistance mechanisms have been demonstrated. There have been more than 17 KD mutations conferring resistance to first generation and/ or next generation ALKi described to date. (31, 63) The frequency of these mutations increases with each line of therapy as the sensitivity to different ALKi varies. For ceritinib, I1123S, L1152R, F1174C/V and G1202R have been identified clinically as resistance mutations. (64) For alectinib, I1171N/T/S and G1202R produce resistance (65); whilst brigatinib is ineffective in the presence of G1202R (31). (64) For alectinib, I1171N/T/S and G1202R confer resistance (65); whilst brigatinib is G1202R sensitive (31). Lorlatinib has demonstrated pan inhibitory activity against the known KD mutations apart from L1198F which paradoxically maintains crizotinib sensitivity. (40)

Although demonstration of the presence of specific *ALK* mutations could establish the sequence of treatment with various ALKi, mechanisms other than mutations in *ALK* drive crizotinib resistance in most tumours. Therefore, the *ALK*-independent mechanisms highlighted in **Figure 4** relate predominantly to resistance to the next-generation TKI.

4.5 Treatment Beyond Progression and Managing Oligoprogressive Disease

The PROFILE trials permitted treatment beyond RECIST progression if prior disease control had been achieved in those still deriving clinical benefit. (23, 25) Local aggressive therapy of both CNS and extra-cranial disease and ongoing crizotinib therapy was also allowed. In the PROFILE 1001 and 1005 trials 62% of patients who had progressed at data cut off remained on crizotinib. In patients who were treated beyond progression the mOS from the time of PD in those who continued crizotinib was 16.4mo versus 3.9mo (p<0.0001). (66)

This concept is supported scientifically as NSCLC represents a complex genomic landscape. Intra-tumoural and inter-tumoural heterogeneity and treatment selection pressure drives clonal diversity fuelling drug resistance.(67)

Further challenges lie in the setting of CNS only progression given drug delivery is sub-optimal with crizotinib. Continuation of targeted therapy when there is no-systemic progression after local CNS therapy has proven beneficial. (68)

Treating a resistant clone with ablative therapy before widespread dissemination may prolong disease control until either a new event occurs or resistant clones that have disseminated expand sufficiently to become detectable. Targeted therapy can continue to be of benefit in other sites of non-progressing disease because of continuing suppression of sensitive clones that have not yet developed acquired resistance. A retrospective series has demonstrated this approach can extend systemic treatment benefit by 6mo. (69) Switching therapy at disease progression whilst concurrently managing the dominant progressing lesion with aggressive local therapy has been reported to potentially enhance an ongoing durable systemic therapy benefit. (70)

Therefore, in clinical practice where there is the presence of 'oligoprogressive disease', for example CNS, local ablative therapy with the continuation of existing systemic therapy, may be a viable approach in selected patients. However, an immediate change of therapy should be the preferred strategy in patients with significant and symptomatic progression.

4.6 Use of Immunotherapy in *ALK* NSCLC

At present there is no evidence to support the use of single agent immunotherapy in *ALK* rearranged NSCLC. The phase III CheckMate-057 included only three patients with *ALK* rearrangements, however there were 53 cases with *EGFR* mutations in which there was a trend to improved efficacy with docetaxel chemotherapy. (71) The equivalent phase II/III KeyNote 010 trial with pembrolizumab yielded the same finding. (72) The lack of benefit was also seen in non-smokers who represent a great proportion of those with *ALK* rearrangements. Clinical trials have since excluded patients with *ALK* rearrangements.

PD-L1 expression is however found to be upregulated by the *EML4-ALK* by activating downstream PI3K-AKT and MEK-ERK signalling pathways. (73) A small series has found PD-L1 expression is five times higher in *ALK* translocated patients than in wild-type patients. (74) Studies are underway to review combination therapies encompassing immunotherapy. Early data from a phase I dose escalation trial of ceritinib with nivolumab have demonstrated activity; however, the toxicity profile warrants review before further investigation (NCT02393625). (75)

4.7 Treatment Sequencing of *ALK* Inhibitors

It remains unknown how to best sequence ALKi therapy. Therapeutic decision making to date has been dictated by Phase III clinical trial data and drug access limitations. **Figure 5** provides a current treatment algorithm for treating ALK+ NSCLC, albeit predicted to evolve, particularly following on from the Phase III alectinib data.

In the PROFILE 1007 study mOS in patients who crossed over to crizotinib from chemotherapy from the time of cross-over was 20mo, was far superior to prior NSCLC reports. (25)

Retrospective real world data are becoming available on the experience with treatment sequencing. One such report of 73 patients treated with crizotinib followed by ceritinib produced a combined mPFS of 17.4mo. (76)

A similar small series investigated crizotinib with alectinib in only 11 patients and delivered a comparable combined mPFS of 18.2mo. (23, 77) An Italian series reported an ORR with second line ALKi of 86.4%, analogous with clinical trial data. (78)

The most impressive experience to date is from a large retrospective French series of 318 patients treated with crizotinib in different lines that found in 84 patients who went on to receive a next generation ALKi, a median post- crizotinib progression OS of 25.0mo and mOS for all patients of 89.6mo. (79)

As is our experience with next line treatment across tumour types, early phase lorlatinib data revealed an attenuated response to lorlatinib when utilised in the third line versus second line setting (mPFS 9.2mo compared to 13.5mo). (40) Whether to use this most potent ALKi upfront or to reserve it is a question requiring further exploration. Patient factors such as performance status and tumour burden may further guide this decision, as reserving drug may not be beneficial for all. (80)

Recent data from the first line second generation ALKi alectinib phase III trial revealed alectinib treated patients had not reached mPFS after 18.6mo follow up. It is predicted to surpass the cumulative mPFS (across trials) for first generation crizotinib with second generation TKIs in the second line setting offering a compelling argument to utilise the most potent ALKi upfront. (46)

5. Future Directions

5.1 Importance of repeat biopsies for emergence of drug resistance

Understanding the temporal genetic changes influencing drug resistance may guide the selection of further tailored treatments in those patients affected. It is widely encouraged and accepted that tumour biopsies need to be performed at the time of disease progression if feasible. The risks and benefits of re-biopsy have been discussed and supported in the literature and are of pivotal importance in *ALK*+ NSCLC. (81)

The recent landmark case report of an *ALK*+ NSCLC patient who had multiple repeat biopsies at tumour progression to potentially guide therapy emphasises the value of repeat biopsy. (82) Thirty-six months into treatment, the patient developed a new KD mutation (L1198F) implying lorlatinib resistance. Remarkably, the patient was rechallenged with crizotinib and achieved durable response despite prior resistance. (82)

It remains unclear if a particular sequence of ALKi influences the emergence of specific resistance mechanisms, and therefore the clinical course of the patient. Therefore, the question of the most appropriate sequence of ALKi for the individual patient can only be answered by trials such as the proposed ALK MATCH trial, a concept similar to the ongoing

NCI-MATCH trial identifying genomic drivers in cancer in order to develop more effective treatment approaches (NCT02465060).

5.2 Liquid biopsies for less-invasive identification of mechanisms of resistance

Plasma circulating free DNA (cfDNA) enabling mutational profiling may be a less-invasive, easily accessible tool for the detection of molecular changes and *ALK* rearrangements, including secondary mutations. (60). This could be used to identify impending resistance mechanisms thereby facilitating early treatment change and optimal ongoing therapeutic choice although this approach requires further prospective studies to confirm utility.

The challenge of using cfDNA for ALK is similar to those described above where PCR amplification of the target sequence is needed to detect the rearrangement. Currently this requires either developing an individualised assay for each breakpoint or using a pre-designed assay with multiple breakpoints.

Reports on sensitivity and concordance with liquid assays compared to tissue biopsy have been published as a validated commercial assay for 'liquid biopsy' is yet to be available. (61)

Circulating tumour cells (CTCs) may also represent a non-invasive and easily accessible source of tumour material for assessing predictive molecular biomarkers and screening patients eligible for targeted treatments. Recently, a modified FISH assay with filtration enrichment (FA-FISH) was developed to identify *ALK* rearrangements using CTC in *ALK*, which may allow for a diagnostic and monitoring presence in the future as a non-invasive predictive biomarker. (83)

With increasing accessibility and cost effectiveness NGS is predicted to become the gold standard of resistance testing in both solid and liquid samples in ALK.

5.3 Accessibility of ALKi

The predicted economic cost in facilitating the availability of multiple ALKi in the clinic, as well companion diagnostic panels, is a further pivotal consideration which will influence how we manage *ALK* NSCLC in the future. Not only is our management directed by the updated literature but also our ability to access treatment.

5.4 Adjuvant ALKi

Investigation is ongoing in to the efficacy of ALKi over chemotherapy in the adjuvant setting. The ALCHEMIST trial of crizotinib in patients with stage IB-IIIA resected NSCLC is currently recruiting (NCT02201992). There has been a suggested disease free survival benefit of TKI therapy (gefitinib) in a preceding trial in the EGFR adjuvant setting, with OS data awaited. (84)

6. Conclusions

The treatment landscape in *ALK*-rearranged NSCLC has rapidly progressed over the past decade and continues to evolve. The evidence for current management is based around pivotal Phase III clinical trial data. However, with multiple drugs and multiple trials, many questions remain regarding the optimal therapy and drug sequence.

Future patient management is predicted to include drug resistance profiling to enable more personalised drug treatment with ALKi as the potential for combination treatments, particularly ALKi with immunotherapy are investigated.

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Tables

Agent	First Line *mPFS and Trial Phase	Second Line mPFS and Trial Phase
Crizotinib	10.9 mo (III) (23)	7.7 mo (III) (25)
Ceritinib	16.6 mo (III) (35)	6.9 mo (II) (27)
Alectinib	25.9 mo (III) (37)	8.9 mo (II) (30)
Brigatinib	Not available	13.2 mo (I/II) (38)
Entrectinib	Not available	8.3 mo (I) (34)
Lorlatinib	Not available	13.5 mo (I/II) (40)
Chemotherapy	7.0 mo (III) (23)	3.0 (III) (25)

Table 1. Systemic median progression free survival on ALK inhibitors available commercially, registered by governing pharmaceutical bodies or under clinical trial evaluation with current results available. *mPFS= median progression free survival in months (mo)

Agent	First Line *mPFS and Trial Phase	Second Line mPFS and Trial Phase
Crizotinib	9.0 mo (III) (41)	Not reported
Ceritinib	10.7 mo (III) (35)	5.2 mo (II) (28)
Alectinib	Not reached (46)	10.3 mo (DOR) (II) (30)

Brigatinib	Not available	Not reported
Entrectinib	Not available	Not available
Lorlatinib	Not available	Not reported
Chemotherapy	4.0-6.7 mo (III) [35, 41]	Not reported

Table 2. CNS median progression free survival on ALK inhibitors available commercially registered by governing pharmaceutical bodies or under clinical trial evaluation with current results available. *mPFS= median progression free survival in months (mo)

Figures

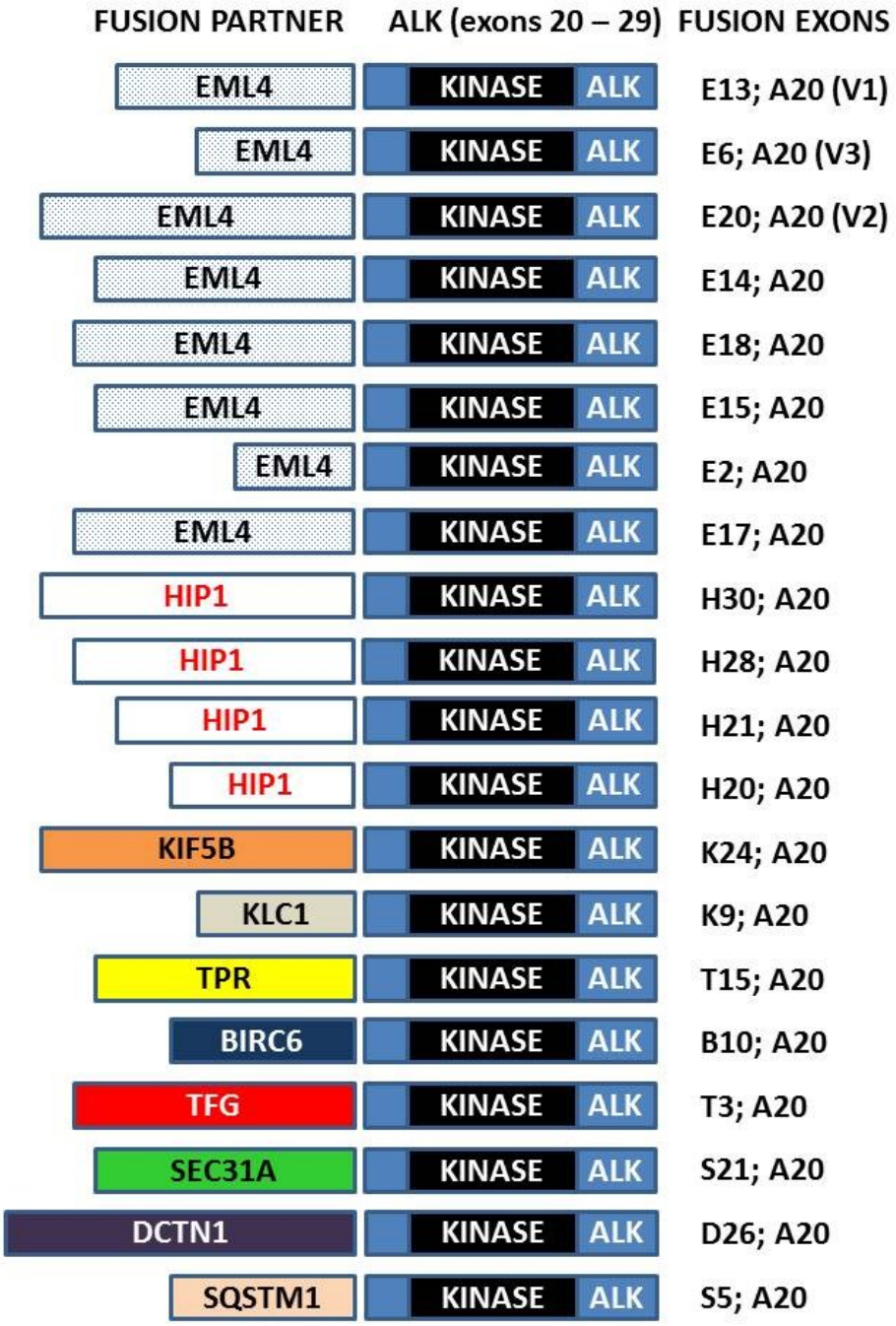


Figure 1 ALK fusion genes reported in NSCLC. All fusion genes contain *ALK* exons 20-29 which includes the Tyrosine Kinase domain. Fusion Exons indicate the exons at which the breakpoints / fusion of each gene occur, eg E13; A20 indicates a fusion between *EML4* exon 13 and *ALK* exon 20. V1 is the most common fusion in NSCLC followed by V3 and V2. *HIP1*: Huntingtin-interacting protein 1, *KIF5B*: kinesin family member 5B; *KLC1*: kinesin light chain 1; *TPR*: Translocated promoter region, nuclear basket protein; *BIRC6*: and baculoviral inhibition of apoptosis protein repeat containing 6; *TFG*: TRK-fused gene; *SEC31A*: SEC31 homolog A, COPII coat complex component; *DCTN1*: dynactin subunit 1; *SQSTM1*: sequestosome 1.

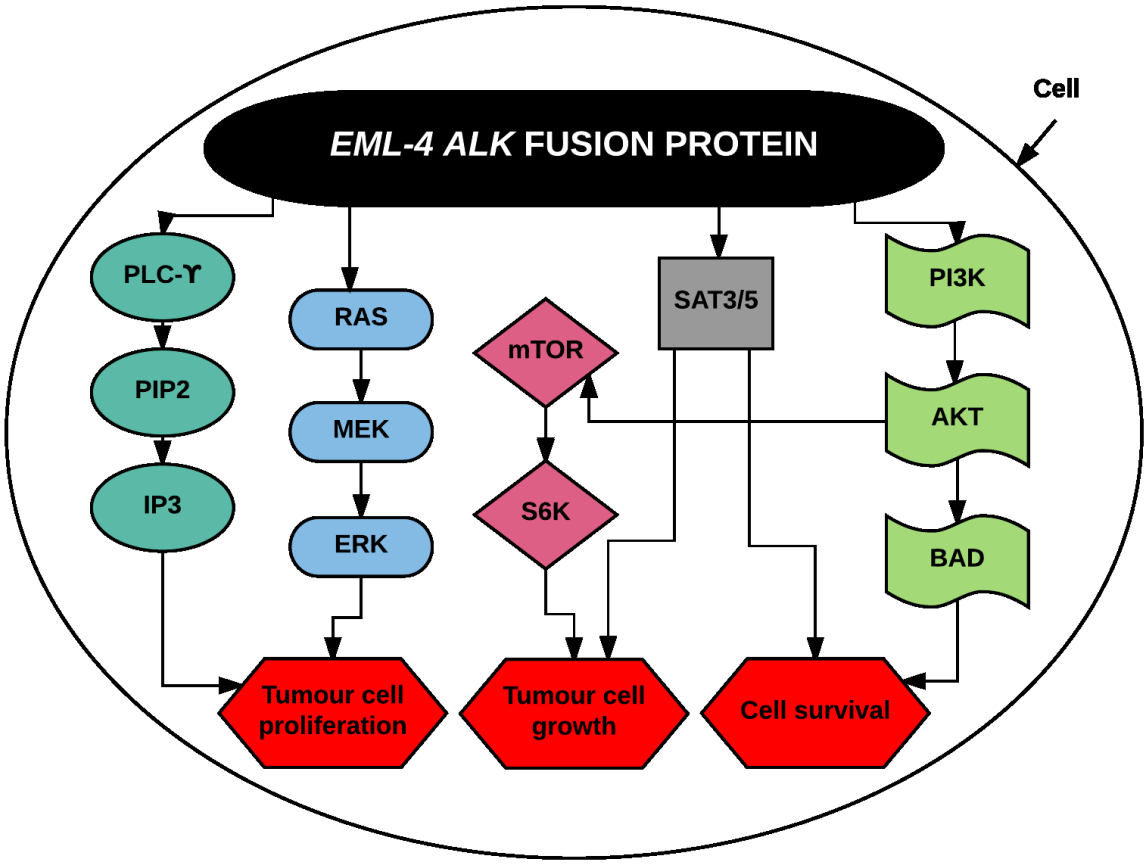


Figure 2. Intracellular enzymatic cascade due to the aberrant *EML4-ALK* fusion oncogene Adapted from (8)

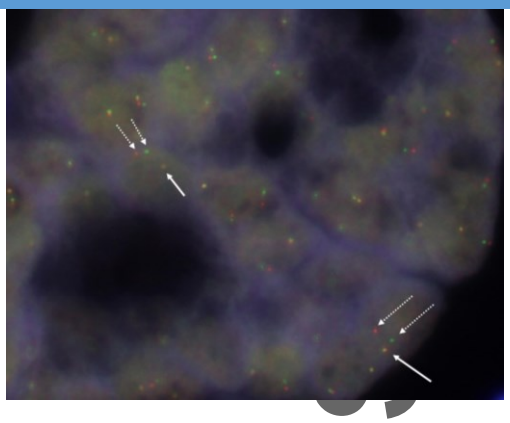
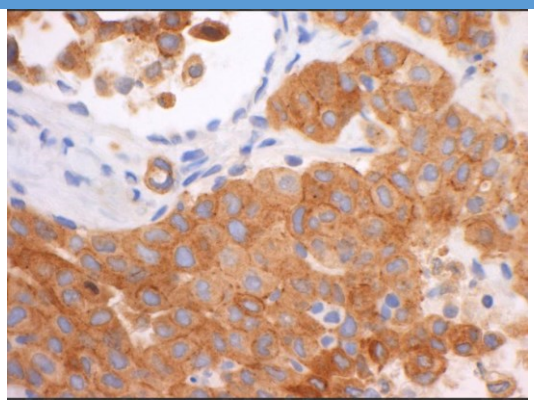
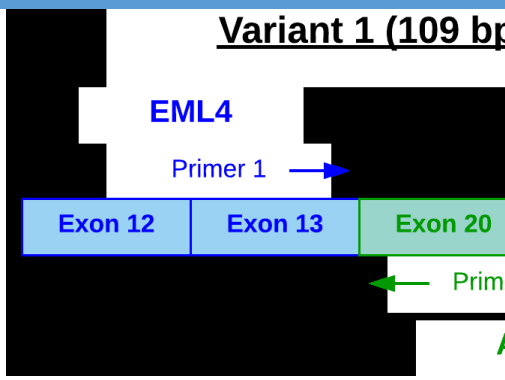
FISH	IHC	RT-PCR
		
• Gold standard assay • Very good specificity • Automated, high-throughput workflow available for *ALK* • Can be performed in small biopsy samples • Required for Australian PBS subsidy	• Excellent (100%) sensitivity* • Rapid-throughput method using automated platforms • Accessible in virtually all Australian centres • Can be performed in small biopsy samples • Tissue morphology is retained during analysis	• High sensitivity • Can detect rearrangement and fusi • High specificity for known fusion pa • Can be applied to biofluids (e.g. blo effusion, bronchial wash)
• Relatively low sensitivity • FISH signal instability • Result interpretation can be subjective- requires expert interpretation • Need at least 100 tumour cells • Only available in specialized centres	• Moderate specificity* • Requires clinical validation to become “gold standard”	• Cannot detect novel fusion partner • Requires assay design and optimis novel fusion partner identified • Requires high-quality RNA (difficult small FFPE specimens) • Cross-contamination between spec occur
$$$	$	$$

Figure 3. Comparison of routine diagnostic assays for *ALK* NSCLC: (A) FISH analysis of a lung adenocarcinoma sample showing cells with an *ALK* translocation using a break apart probe. In this method, two differently coloured probes (red and green) flank the highly conserved translocation breakpoint within *ALK*. Separation of the probes due to an *ALK* rearrangement results in separation/splitting of the red and green signals in the affected chromosome (dashed arrows). In the other normal chromosome, the overlying red and green probes result in a yellow (fused) signal (solid arrow); (B) An example of ALK IHC (variant 1) positive staining. IHC involves selectively imaging antigens (in this case, ALK protein) in

cells of a tissue section using specifically designed antibodies. Tissue sections are scored positive if strong granular cytoplasmic staining (dark brown colour) in tumour cells are present; (C) RT-PCR: In RT-PCR, RNA is reverse transcribed (RT) to complementary DNA (cDNA) prior to amplification by polymerase chain reaction (PCR) with primers flanking the breakpoint and fusion. A product will be obtained only if that particular fusion gene is present.

*Sensitivity and specificity of the different techniques were compared in (85); benefits/cost compared in (86)

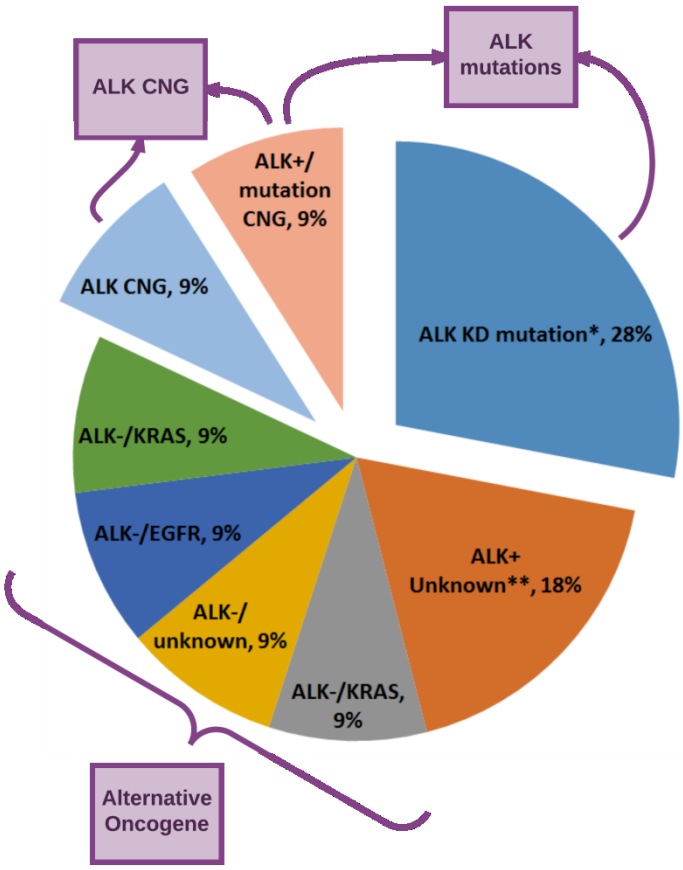


Figure 4. ALK-Dependent and ALK-Independent mechanisms of resistance to crizotinib. *Key examples of ALK kinase domain mutations conferring crizotinib resistance include: L1196M, G1202R, L1152R, C115Y, and F1174L. **ALK+ Unknown category may also include not yet identified alternative ALK-dependent mechanisms of resistance. CNG: copy number gain; KD: kinase domain; ALK−: loss of dependence on the ALK pathway; EGFR: epidermal growth factor receptor; KRAS: Kirsten rat sarcoma viral oncogene. Adapted from: (10, 50)

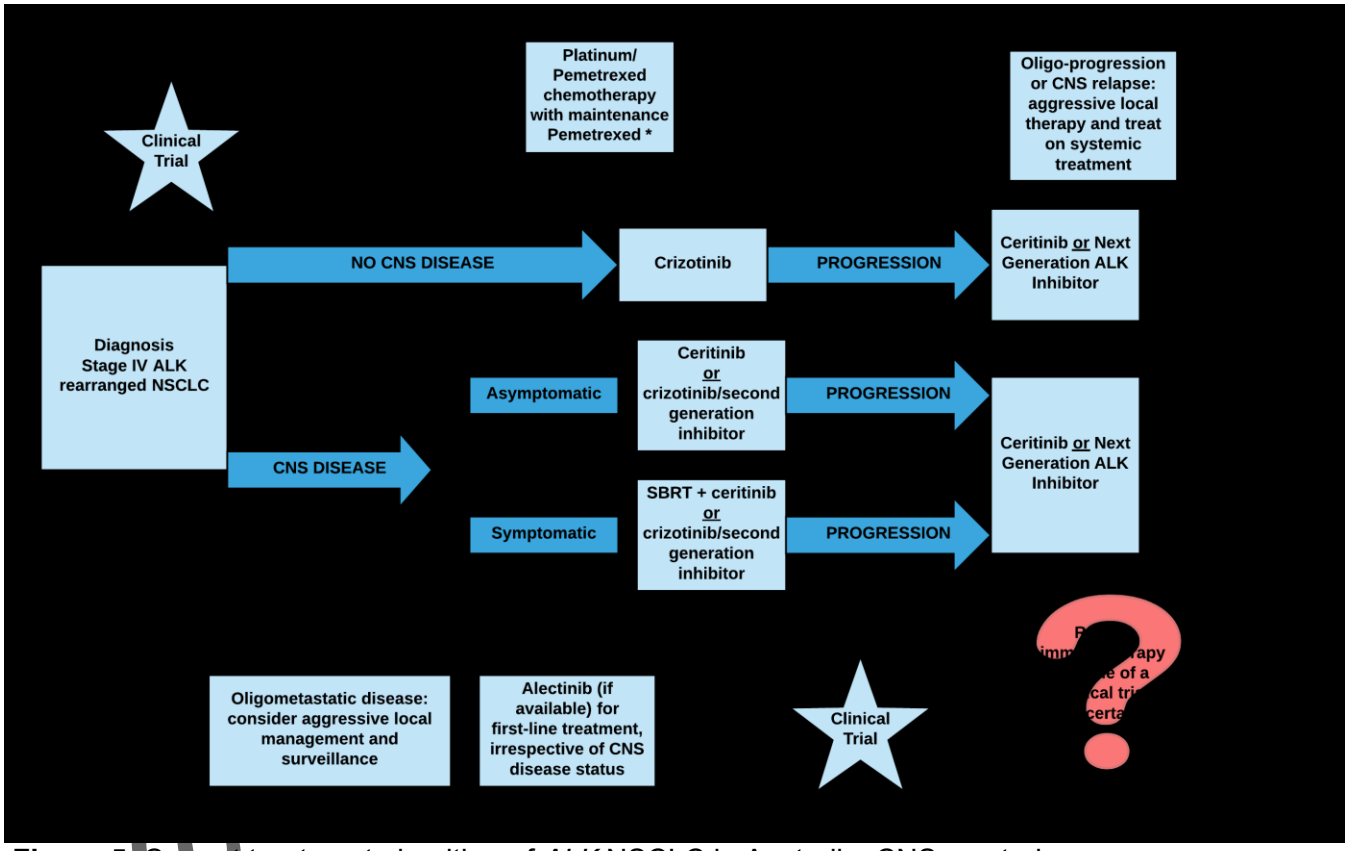


Figure 5. Current treatment algorithm of *ALK* NSCLC in Australia. CNS: central nervous system; SBRT: Stereotactic Body Radiation Therapy; WBRT: Whole brain radiation therapy.

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