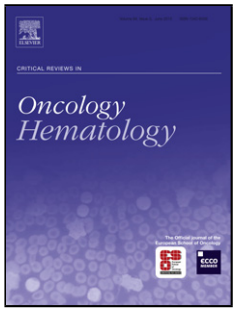
Precision medicine in ALK rearranged NSCLC: A rapidly evolving scenario



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Precision medicine in ALK rearranged NSCLC: a rapidly evolving scenario

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Introduction

Deeper understanding of the pathobiology of non-small cell lung cancer (NSCLC) has led to the development of small molecules that target genetic mutations known to play critical roles in the progression to metastatic disease. Mutations in epidermal growth factor receptor (*EGFR*), kirsten ras sarcoma oncogene (*KRAS)* and anaplastic lymphoma kinase (*ALK*) translocations are generally mutually exclusive in patients with NSCLC and the presence of one alteration in lieu of another can influence responses to targeted therapy. Thus, testing for these mutations and tailoring therapy accordingly is widely accepted as standard practice[1,2,3].

ALK gene rearrangement in NSCLC was identified for the first time in a resected adenocarcinoma specimen from a 62-year-old male smoker. Rearrangements, either inversions or translocations, characterize the genomic disruptions involving *ALK* observed in NSCLC[4,5]. Inversions in the short arm of chromosome 2 that juxtapose echinoderm microtubule–associated protein-like 4 (*EML4*) with *ALK* and produce *EML4-ALK*–fusion tyrosine kinases[4,6] are the most common noted changes but at least 27 fusion variants have been identified[7]. The reported prevalence of *ALK* rearrangements in unselected NSCLC is approximately 5%[8,9]. Remarkably, tumors with *ALK* rearrangements are addicted to ALK signalling and are inhibited by ALK Tyrosine Kinase Inhibitors (TKIs) in preclinical models[10,11,12].

In the past years several ALK inhibitors (ALKi) have been developed and become widely available in clinical practice; they are listed in table 1 with indication/approval along with the registration trials. Despite the efficacy of all these drugs, all ALK+ lung cancer patients will inevitably progress at some point during their treatment. To date, we are aware of two major mechanisms of resistance: ALK-dependent (primary resistance, secondary acquired mutations, gene amplification) and ALK-independent (by-pass signalling, drug efflux pump, epithelial-to-mesenchymal transition). Mechanisms of primary resistance are poorly understood and the spectrum of known secondary mutations mirrors Chronic Myeloid Leukemia (CML) and its mutational landscape acquired during imatinib treatment[13].

Unfortunately, the initial clinical response to targeted kinase inhibitors is almost always temporary, as acquired resistance to these drugs invariably develops. Many mechanisms of resistance to each targeted therapy have been identified, but can be generally categorized into two predominant classes: (1) alteration of the driver oncogene, (2) activation of a critical signalling pathway(s) in a parallel or downstream fashion, driving pro-survival signalling through different pathways.

The most common and well established mechanism of resistance for the EGFR is the alteration of the driver oncogene, where the gatekeeper T790M mutation is found in ~50% of EGFR-mutant patients who become resistant to EGFR inhibition[14,15] This has led to the development of several third-generation EGFR inhibitors, that could potentially block the growth of *EGFR* T790M-positive tumors[16,17,18]

Unlike EGFR, type and frequency of ALK resistant mutations changes based on the inhibitor class. In crizotinib-refractory patients the most frequent mutations are L1196M and G1269A. The first is a classical gatekeeper mutation that alters the catalytic domain and causes resistance to ATP-competitive inhibitors[19], as in EGFR-T790M+ lung cancers. The latter, G1269A, determines a steric hindrance impairing the proper binding of crizotinib[20]. A plethora of less frequent mutations have been also described such as C1156Y, L1152R, 1151 T-ins at the N-terminus domain, I1171T, F1174L near the activation loop and G1202R, S1206Y in the solvent-exposed region close by the crizotinib binding-site[21,22,23,24]. Patients progressing on crizotinib treatment, regardless the presence of acquired mutations or not, seemed to be still ALK-dependent, as they respond to next-generation inhibitors, probably due to the limited ALK-blockade potency of crizotinib[25].

In large biopsies series from ALK+ NSCLC treated patients, the number of detected mutations increased after second generation ALKi[26] and in one study were present in 56% of the entire cohort.[42] For example, the rate of G1202R mutations increases from 2% in post-crizotinib treated patients to 43% in post-brigatinib cases highlighting a specific mutational profile associated to each

ALK TKI. The C1156Y mutation is less efficiently inhibited by ceritinib, contrary to the I1171T[41] mutation, identified in post-alectinib samples, that results sensitive to ceritinib. The gatekeeper mutation L1196M is inhibited by alectinib but emerges as a post-alectinib mutation itself; F1174L mutations determine resistance to ceritinib but are still sensitive to alectinib; the G1202R, most common mechanism of resistance post-second-generation ALKi (ceritinib, alectinib, brigatinib), is efficiently inhibited only by the third generation compound, lorlatinib, in preclinical models and patients[42,]. This scenario becomes even more complex if we add the presence of compound-related resistance mutations that emerge in patients treated with sequential ALK inhibitors: tumor clones harbouring E1210K/D1203N mutations after crizotinib and brigatinib remains sensitive only to lorlatinib[42]; on the other hand, a double mutant patient (C1156Y/L1198F) resistant to crizotinib, ceritinib and lorlatinib appeared to regain sensitivity to crizotinib, with a durable response[27].

If ALK gene amplification has been identified as resistance mechanism only in a small fraction (9%) of crizotinib refractory cases, multiple by-pass signalling tracks, which account for ≈40% of non-mutated patients refractory to second-generation ALKi[28], have been described: EGFR and HER family members activation[29], also triggered by paracrine stimuli[30], MET amplification [19], activation of downstream signalling pathway (i.e. RAS-MEK), even by specific MAP2K1 mutation that makes cancer cells sensitive to ALK/MEK co-inhibition[31], c-KIT amplification requiring SCF [6], IGF-1R upregulation[32] SRC activation[47] and engagement of P2Y receptors[33]. Notably, efflux (MDR1 encoded) pump over-expression may be considered an alternative mechanism of resistance, as demonstrated in patients treated with crizotinib and ceritinib[34], whose CNS penetration is hindered compared to alectinib that is not a substrate of this drug efflux system.

Lastly, transition to a mesenchymal phenotype represents an alternative escape strategy. EMT has been described in post-ceritinib samples[42] although the real contribution and underlying molecular mechanisms have not been elucidated yet. Some hypothesis came from the similar scenario of EMT in EGFR-mutant NSCLC in which alternative activation of AXL, IGF-1R or the SRC/FAK pathways have been proposed as causative molecular events[35,36,37].

ALKi testing, sequencing and best strategies

The general consensus of the ATLAS IALSC Guidelines[38] is that screening for *ALK* gene rearrangement should be performed for all patients with advanced NSCLC, mainly adenocarcinoma or with adenocarcinoma component. Depending on resources and academic interest, screening of patients with advanced NSCLC of other histologies should be considered, especially patients with one or more of these features: younger patient age, never/light smoking history, or negative results on testing for *EGFR* and *KRAS* mutations. *ALK* gene rearrangement may be found in tumors with non-adenocarcinoma histologies, although this finding is rare[39,40].

A re-biopsy at progression remains a practice extremely heterogeneous and not completely codified despite the fact that, if a secondary mutation is identified at progression, the tumour should be considered still ALK-dependent and the appropriate ALKi (figure 1) offered, based on the mutational sensitivity. This approach might give the opportunity to further control the cancer, delaying the use of the standard chemotherapy.

It appears evident that the therapeutic landscape has been rapidly evolving and the future of ALK+ NSCLC treatment is promising with multiple therapeutic options over the past years as summarised in table 1.

Crizotinib was the first ALK inhibitor that showed a benefit over the standard chemotherapy treatment in term of increased PFS in the second line setting, compared to chemotherapy in the Profile 1007[41] and in first line setting n the Profile 1014[42] compared to standard platinum based chemotherapy.

Subsequently a different Alk inhibitor, Ceritinib, was developed and tested in several trails either in first line, similarly to the Profile 1014[42] design, within the Ascend 4[43] where ceritinib proved to be superior in term of PFS compared to the standard first line chemotherapy treatment, either after progression to chemotherapy and to crizotinib in the Ascend 5[44]

Notably J-Alex trial first[45] and the Alex trial[46] later have showed the superiority of alectinib over crizotinib in term of PFS and time to central nervous system (CNS) progression in first line for this group of patients

The J-ALEX trial, the first randomized phase III trial to directly compare two ALK inhibitors (alectinib versus crizotinib) in the first line setting[41], was conducted exclusively in Japan at 41 study sites between November 2013 and August 2015 and 207 patients with stage IIIB/IV ALK positive NSCLC, who had previously received 0–1 lines of chemotherapy, but no prior ALK TKI, were enrolled and randomized to alectinib 300 mg twice daily or crizotinib 250 mg twice daily. At the time of planned interim analysis, median PFS was not reached in the alectinib arm (20.3 months at the low end of the CI) and was 10.2 months in the crizotinib arm (HR 0.34, 99.7% CI 0.17–0.70). The ORR of alectinib in the intention to treat population was 85.4% (95% CI 78.6–92.3) versus 70.2% (95% CI 61.4–79) in the crizotinib arm. In the subgroup of patients with brain metastasis, there was also a strikingly improved response to alectinib (HR 0.08, 95% CI 0.01–0.61). For patients with brain metastatic lesions at baseline, the HR for the time to progression of a brain metastatic lesion or death was 0.16 (95% CI 0.02–1.28), and for patients without brain metastatic lesions at baseline, the HR for the time to onset of a brain metastatic lesion or death was 0.41 (95% CI 0.17–1.01). All grade adverse events favored alectinib with the most common side effects in the alectinib arm.

The results of this study were considered certainly compelling with some possible drawbacks: relatively large percentage of patients pre-treated with chemotherapy and a significantly larger percentage of patients with brain metastasis in the crizotinib arm compared to the alectinib one.

The ALEX trial results were presented at ASCO 2017 with simultaneous publication in June 2017. It was an international phase III trial launched across 161 locations in 31 countries, with 303 treatment naïve ALK positive metastatic NSCLC patients randomized to alectinib 600 mg twice daily or crizotinib 250 mg twice daily, with PFS again being the primary endpoint [42]. Secondary endpoints included time

to CNS progression, ORR, DOR, OS, quality of life, and safety. After a follow up of 17.6 months in the crizotinib arm and 18.6 months in the alectinib arm, median PFS was not reached in the alectinib arm versus 11.1 months with crizotinib (HR 0.47, 95% CI 0.34–0.67, P<0.001). The effect was seen across nearly all subgroups with the exception of smokers and patients with an ECOG of 2, though these represented small numbers of patients. Time to CNS progression was also significantly longer with alectinib, with a 12-month incidence rate of CNS progression of 9.4% (95% CI 5.4–14.7) with alectinib versus 41.4% (95% CI 33.2–49.4) in the crizotinib arm. Among those patients with measurable CNS metastasis at baseline, 81% (95% CI 58–95) had a response in the alectinib arm versus 50% (95% CI 28–72) in the crizotinib arm, with 38% in the alectinib arm having achieved a complete response.

The Alex trial was slightly different from the J-Alex: the study population included patients from multiple countries, the dose of alectinib used was 600 mg twice daily, all the patients were treatment naive, whereas those in the J-ALEX trial could have received chemotherapy initially. However the results of both trials closely mirrored each other and clearly demonstrated that in the frontline setting, alectinib is superior to crizotinib in terms of PFS, ORR, CNS response, and tolerability.

Could we conclude, based on the evidence we have so far, that alectinib should be regarded as the new standard of care in first line or a sequential approach consisting of crizotinib first followed by alectinib could still be a preferable option? Despite the fact that the OS data for both, J Alex and Alex trial, are not yet available, the magnitude of PFS benefit seems to suggest that using the more active drug, alectinib, up front could guarantee a better outcome particularly for its CNS activity and efficacy.

Furthermore several ALKi (lorlatinib[47], brigatinib[48]) have recently showed impressive results on naive and pre-treated ALK+ NSCLC patients increasing the number of therapeutical option available other than crizotinib in phase I-II trials. Thus, if the first line treatment seems to be relatively clear, the question “What to do next?” has become more than ever important and defining the optimal treatment strategy is the new task for the scientific community, even more than developing new ALKi. In the contest of correct sequencing, even if in a different clinical and genomic scenario, a possible answer might come from the APPLE-EORTC1603 trial[49]: a randomized, open-label, multicenter, 3-arm, phase II study in advanced, EGFR-mutant and EGFR-TKI-naive NSCLC patients, to evaluate the best strategy for sequencing gefitinib and osimertinib treatment. In all arms, a plasmatic ctDNA T790M test will be performed and the primary objective will be to evaluate the best strategy for sequencing of treatment with gefitinib and osimertinib in advanced NSCLC patients with common *EGFR* mutations, and to understand the value of liquid biopsy for the decision-making process. Even if on a different contest, the EGFR mutated patients, the result might point out that using the most effective TKI, based exclusively on the increased PFS, might not be the best strategy and that sequencing carefully and properly the different and extremely active TKIs has to be carefully considered and it might offer a longer OS in the end.

Although ongoing and future trials will be trying to establish the correct sequencing, we’d want to propose, based on the drugs development, the knowledge on mechanisms of primary and secondary resistance mutations that we already have, a possible treatment algorithm for ALK rearranged NCSLC

patients (figure1) stratified according to mutations detected throughout their clinical story by serial tissue or liquid biopsies performed at progression.

Upfront and at relapse TKI combination might represent a valid strategy to delay or counteract, once appeared, ALK-independent by-pass track signalling pathways. Moreover permits to delay on-target resistance mechanisms, reducing the typical clonal pressure of single potent TKI monotherapy, like appearance of compound resistance mutations. Potential augmented toxicities of combined TKI politherapies may represent a major issue, partly curbed by reduced dosages or alternative drug schedules. Different strategies have been considered; EML4-ALK fusion proteins are known client of HSP90 chaperone machinery and therefore ganetespib demonstrated efficient control of ALK+ NSCLC either in presence or absence of ALK secondary mutations [54]. Even if not totally understood, this may be related to the wider HSP90 range of activities; parallel TRK (e.g. HER2) pathways, which sustain ALK+ cancer cells, are targeted by HSP90 and its inhibition contribute to shut down downstream signalling pathways. HSP90i alone or in combination with ALKi have been investigated (NCT01752400, NCT01712217). Powerful association of pan-HER and ALK inhibitors, supported by strong preclinical evidences[50], had been limited by high-grade adverse events . ALK/MEK dual inhibition represents a promising therapeutic tool in order to up-front delay resistance mechanisms and improve response duration [56]. Also, MEK mutations appear in a ceritinib-treated patient and MEK inhibition contributes to disease control. Trametinib/ceritinib association is under evaluation (NCT03087448). MET amplification has been identified as post-alectinib resistance mechanism and thus the specific patient responded to crizotinib treatment .

Unfortunately, day to day practise might be, by far, different and more complex than what we have described here. In many European countries and in the United States of America the ALK treatment pathway is guided by labelling system and many ALKi are simply not available (table 2). Other than legislation issues and regulatory limitations, to make our algorithm even more challenging, is the fact that performing a re-biopsy at progression in many countries and institutes is not always possible or straight forward given the lack of staff, funding and facilities. Furthermore, even if the drugs were available, there isn't consensus on the specific mutations to look for at progression, which makes even a re-biopsy possibly academic or not effective as it could be.

At certain point all the ALK+ NSCLC will become ALK-independent and in these cases the patients could not benefit from another ALK inhibitors but standard chemotherapy[51] or combination strategies ought to be considered.

Future studies investigating alectinib-based combinations are already underway including combination with the programmed cell death ligand 1 (PD-L1) inhibitor atezolizumab and or bevacizumab. Integration with Immunotherapy (IO) seems limited by the fact that ALK+ tumors arose in patients with a low mutational load and PD-L1 and CD8 expressions are underrepresented [58]. Studies of these and other alectinib-based combinations should help to identify new therapeutic strategies that can overcome and even potentially prevent resistance. Patients lacking any traceable alteration or clinical-useful biomarker may be recruited for platinum-pemetrexed chemotherapy since ALK+ NSCLC appeared to be particularly sensitive to these therapies: pemetrexed association with crizotinib is object of current clinical evaluation (NCT02134912).

Conclusions

Despite the improvement in the knowledge of resistance mechanisms and the efficacy of several ALKi, there are still hurdles to overcome: drug costs and/or local legislation narrow and limit the treatment option for this group of patients. Stronger collaborations between academia, pharmaceutical companies and regulatory authorities need to be spurred to implement availability and affordability of such drugs.

Clinical trials are warranted to further investigate ALKi sequencing with a great interest to the EORTC 1603 trial, to further understand the emerging resistance mechanisms after first-line alectinib and to develop possible strategies for delaying and overcoming these mechanisms.

In conclusion we have summarized the evolution and improvement of ALK+ NSCLC patients treatment and highlighted, in this group of patients, a possible customized strategy, which would be potentially applicable and would represent a step towards personalized medicine.

Table 1 (ALKi trials)

Drug name	**Study**	**Phase**	**Population**	**vs**	**ORR**	**IC-ORR**	**PFS**	**OS**
Crizotinib	PROFILE 1007	III	Platinum-based chemotherapy pretreated (n = 347)	Pemetrexed or docetaxel	65% (95% CI 58–72%) *versus* 20% (95% CI 14–26%;	NA	7.7 *versus* 3.0 months (HR, 0.49; 95% CI 0.37–0.64; $p < 0.001$)	20.3 (95% CI 18.1–not reached) *versus* 22.8 months (95% CI 18.6–not

					$p < 0.001$)			reached) (HR, 1.02; 95% CI 0.68–1.54; $p = 0.54$)
Crizotinib	PROFILE 1014	III	Previously untreated (n = 343)	Platinum plus pemetrexed	74% (95% CI 67–81%) *versus* 45% (95% CI 37–53%; $p < 0.001$)	NA	10.9 *versus* 7.0 months (HR, 0.45; 95% CI 0.35–0.60; $p < 0.001$	Median OS was not reached in either group (HR for death with crizotinib, 0.82; 95% CI 0.54–1.26; $p = 0.36$)
Ceritinib	ASCEND 4	III	Previously untreated (n= 376)	Platinum plus pemetrexed	(72·5% [95% CI 65·5–78·7]) vs (26·7% [20·5–33·7])	72.7% vs 27%	16.6 vs 8.1 months (HR 0.49; 95% 0·37–0·64] p<0·00001	NA
Ceritinib	ASCEND 5	III	Platinum-based chemotherapy and crizotinib pretreated (n= 231)	Pemetrexed or docetaxel	39% vs 7%	35% vs 5%	5·4 vs 1.6 months (HR 0·49 [95% CI 0·36–0·67]; p<0·0001	18.1 vs 20.1 months not statistivaly significant.
Ceritinib	ASCEND 8	I	3 cohorts (267 pts in total), 121 treatment naive	450 mg vs 600 mg vs 750 mg (SOC)	78%, 75% and 70%	NA	15 months PFS rate was 66.4%, 58% and 41%.	NA
Alectinib	Global study	II	Crizotinib preteated	Single arm	50% (95% CI, 41% to 59%)	50%	8.9 months (95% CI, 5.6 to 11.3 months)	NA
Alectinib	ALEX	III	Previously untreated (n= 303)	Crizotinib	82.9% (95% CI 85%-76%) vs 75.2 (95% 67.8%-82.1% p < 0.01)	81% vs 50%	25.7 vs 10.4 months (HR=0.50, 95% CI, 0.36-0.70; p<0.0001)	NA
Alectinib	J-ALEX	III	Previously	Crizotinib	85% (95%	80% vs	20.3 vs	NA

			untreated		CI 78·6–92·3) vs 70% (61·4–79·0 p < 0.01)	52%	10·2 (HR 0·34 99·7% CI 0·17–0·71, p<0·0001)	
Brigatinib	NCT01449461	I/II	Previously Treated with crizotinib and naive (n= 79)	Brigatinib (30-300 mg)	71% in crizotinib-pretreated and 100% in crizotinib-naive group	53%	13.4 months in pretreated crizotinib	NA
Brigatinib	ALTA	II	Previously treated with crizotinib and/or chemotherapy (n= 222)	Brigatinib 90 g vs 180 mg	48% (90mg), 53% (180mg)	51% and 55%	9.2 and 16.7 months	NA
Lorlatinib	NCT01970865	II	6 cohorts including pts naive (275 in tot)	Lorlatinib	90% (naive)	75% (naive)	NA	NA
Ensartinib	NCT02767804	III	NA	Crizotinib	NA	NA	NA	NA
Entrectinib	NCT02097810	I	NA	NA	NA	NA	NA	NA
TPX-0005	NCT03093116	I	NA	NA	NA	NA	NA	NA

Table 2 (ALki approval)

Active ingredient	**Indication**	**Selection**	**Dose**	FDA approval	EMEA approval	NICE approval
Crizotinib	ALK+ metastatic NSCLC	VENTANA ALK (D5F3) CDx Assay Vysis ALK	250 mg bid	untreated patients	untreated patients	untreated patients
Ceritinib	ALK+ metastatic	VENTANA ALK (D5F3)	750 mg od	untreated patients	crizotinib pretreated	crizotinib pretreated

	NSCLC	CDx Assay Vysis ALK			patients	patients
Alectinib	ALK+ metastatic NSCLC	VENTANA ALK (D5F3) CDx Assay Vysis ALK	600 mg bid	untreated patients	crizotinib pretreated patients	MISSING (no evidence submission)
Brigatinib	ALK+ metastatic NSCLC	VENTANA ALK (D5F3) CDx Assay Vysis ALK	90 mg od for the first 7 days; if tolerated, increase to 180 mg od	intolerant to or progressing on crizotinib	MISSING	MISSING
Lorlatinib	ALK+ metastatic NSCLC	VENTANA ALK (D5F3) CDx Assay Vysis ALK	100 mg od	Breakthrough Therapy designation - intolerant to or progressing on crizotinib	MISSING	MISSING
Entrectinib	ALK+ metastatic NSCLC	VENTANA ALK (D5F3) CDx Assay Vysis ALK	NA	on going	MISSING	MISSING
Ensartinib	ALK+ metastatic NSCLC	VENTANA ALK (D5F3) CDx Assay Vysis ALK	NA	on going	MISSING	MISSING

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Figure 1(treatment flowchart)