Review Article

Implementation of functional precision medicine for anaplastic lymphoma kinase-rearranged non-small lung cancer

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Abstract: Anaplastic lymphoma kinase (ALK)-rearranged kinase drives non-small cell lung cancer (NSCLC) in 3–7% of patients and treatment consists of a broad range of approved inhibitors which are administered sequentially in case of resistance developing invariably after approximately 1 year of therapy. Besides the former standard tyrosine kinase inhibitor (TKI) crizotinib, the second-line ALK inhibitors alectinib, ceritinib, brigatinib, as well as the third-line lorlatinib are approved for the treatment of ALK-positive NSCLC patients. The main challenge is to find individual schemes of ALK inhibitors therapy which provide the best benefit for the patients. A host of ALK fusion partners, rearrangement variants and ALK mutations prevent a direct correlation of ALK alterations and sensitivity to specific TKIs within the framework of genomic precision medicine. However, recurrent ALK-positive NSCLC is an aggressive disease resulting in accumulation of tumor cells in pleural effusions which may be collected for in vitro sensitivity testing in a manner designated as functional precision medicine. Provided that these cells are present in sufficient numbers, they can be exposed to all possible drug candidates and their chemosensitivity tested in short-term proliferation assays. In addition to ALK-directed TKIs, cytotoxic drugs and inhibitors of non-target bypassing pathways may be included in the tests. In contrast to other trials in genome-guided precision medicine, a range of suitable therapeutics is available for modified ALK proteins. In summary, ALK-positive NSCLC with pleural effusions offer a unique model to develop, test and validate concepts of functional precision medicine.

Keywords: Non-small cell lung cancer (NSCLC); anaplastic lymphoma kinase (ALK); tyrosine kinase inhibitor (TKI); cancer precision medicine; pleural effusion; chemosensitivity test

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Introduction

Non-small cell lung cancer (NSCLC) accounts for about 85% of all lung cancer cases and is often detected at an advanced stage with poor prognosis. Systemic therapy consisted of cytotoxic chemotherapy with only a minor improvement in the median overall survival (OS) of less than 1 year from diagnosis (1). However, the discovery of specific genetic alterations in subpopulations of NSCLC patients and the subsequent development of targeted therapy have dramatically changed the outlook of NSCLC patients (2). Besides the more frequent epidermal growth factor receptor (EGFR) mutations, rearrangements of *anaplastic lymphoma kinase* (*ALK*) gene is present in 3–7% of NSCLCs with increased prevalence in adenocarcinoma histology, never-smokers or light-smokers and lower age compared to other lung cancer populations (3-5). ALK is a tyrosine kinase of the insulin receptor family with a physiological function in normal cell proliferation and neurogenesis (6). Following the detection of ALK rearrangement in lymphomas, oncogenic echinoderm microtubule associated protein like 4 (EML4)-ALK rearrangements were reported in NSCLC in 2007 (3). The downstream activity of ALK activation in cancer results in an increased cell proliferation and metabolism,

cytoskeleton remodeling, migration and increased survival (7). Pleiotrophin and midkine are known ligands for this receptor (8). Patients are never-smokers or light smokers (less than 10 pack-years). Tumors generally tend to be more centrally located, and patients often present with advanced disease. Cerebral and hepatic metastases are common, as well as pleural and pericardial effusions which seems to affirm the inherent aggressive nature of this cancer (9,10).

The MET proto-oncogene tyrosine kinase inhibitor (TKI) crizotinib was found to constitute an inhibitor of rearranged ALK and the phase I trial PROFILE 1001 led to the approval of crizotinib by the US Food and Drug Administration (FDA) for patients with advanced ALK-rearranged NSCLC in 2011 (11). Comparison of ALK inhibitors to chemotherapy showed that crizotinib and the second-line ALK inhibitors ceritinib and alectinib prolong the progression free survival (PFS) and reveal a significantly better overall response rate (ORR) compared to chemotherapy in the first line as well as second line treatment regimens (12). Furthermore, the intracranial response rate was better with ALK inhibitors compared to chemotherapy. Overall ALK inhibitors are safe and effective treatment option in ALK-positive NSCLC. Currently, four first- and second-generation ALK inhibitors, crizotinib, ceritinib, alectinib, and brigatinib, as well as the third-line inhibitor lorlatinib are approved for clinical practice and many more are under development (13).

ALK rearrangement

The *ALK* gene was initially discovered in 1994 in anaplastic large-cell lymphoma and in 2007, Soda *et al.* identified the first ALK rearrangement in NSCLC, occurring between this gene and the EML4 implying a large inversion or translocation (3). The *ALK* gene belongs to the insulin receptor superfamily, and encodes for a transmembrane tyrosine kinase receptor, comprising an extracellular domain, a transmembrane segment, and a cytoplasmic receptor kinase segment. The EML4-ALK translocation leads to a driver mutation with high oncogenic activity located in the cytoplasm (*Figure 1*). Whereas the breakpoint of ALK is at the exon 20, many fusion variants are generated by fusion with different breakpoints in the EML4 exons 2, 6, 13, 14, 15, 18 and 20, differing in frequencies from V1 (54.5%), V2 (10%), V3a/V3b (34%), to V5a (1.5%) (14-16). The association between outcomes to specific EML4-ALK variants as potential predictive markers for response is rarely investigated so far (17). A study examined 67 stage IV lung cancer patients with EML4-ALK fusion variants 1, 2, and 3a/3b and concluded that the V3 (3a/3b) variant is associated with more metastatic sites at diagnosis, earlier failure after treatment with first or second line ALK inhibitors, platinum-based chemotherapy, and cerebral radiotherapy, resulting in an inferior OS (16,18). Dimerization of the ALK kinase domains triggers canonical pathways such as MAPK, PI3K/mTOR, JAK-STAT, SHH among others. The fusion protein is under control of the *EML4* gene promotor which results in ALK overexpression and constitutive tyrosine kinase activity (19). Furthermore, more than 19 different ALK fusion partners have been discovered in NSCLC, including EML4, KIF5B, KLC1, and TPR (20). The first drug resistance point mutations identified were C1156Y and L1196M. Subsequently, several other point mutations conferring drug resistance have been identified, including: G1269A, F1174L, 1151Tins, L1152R, S1206Y, I1171T, G1202, D1203N, and V1180L. As a screening test, ALK immunohistochemistry (IHC), which can be performed short term at low cost, requires less effort and expertise than fluorescence in situ hybridization (FISH) which is in general use (21,22). In addition, next-generation sequencing (NGS) identifies *ALK* fusion partner genes, ALK mutations, and non-target alterations (23). The sensitivity of a particular NGS assay is affected by confounding factors such as tumor cell purity (dilution of tumor signal with DNA from normal cells) and presence of multiple clonal tumor cell populations within a given sample (intratumor heterogeneity or clonal diversity). However, whole transcriptome sequencing may not be cost-effective for thousands of patients and longer turnaround times are not conducive for clinical decision-making. For instance, the genetic makeup of metastatic tumors has been shown to differ substantially from the primary tumor (24). Additionally, tumors frequently develop resistance to targeted therapies, due to both acquired *de novo* resistance mechanisms and Darwinian selection of pre-existing resistant subclones. The latter mechanism in particular can lead to rapid development of resistance and disease progression (25,26).

Treatment of ALK-rearranged NSCLC

Crizotinib was the first ALK TKI shown to be superior to chemotherapy in randomized phase III trials revealing significant improvement in ORR with targeted therapy (65% *vs.* 20%) (27,28). Thus, crizotinib was established as a new standard of care in second- and first-line therapy,

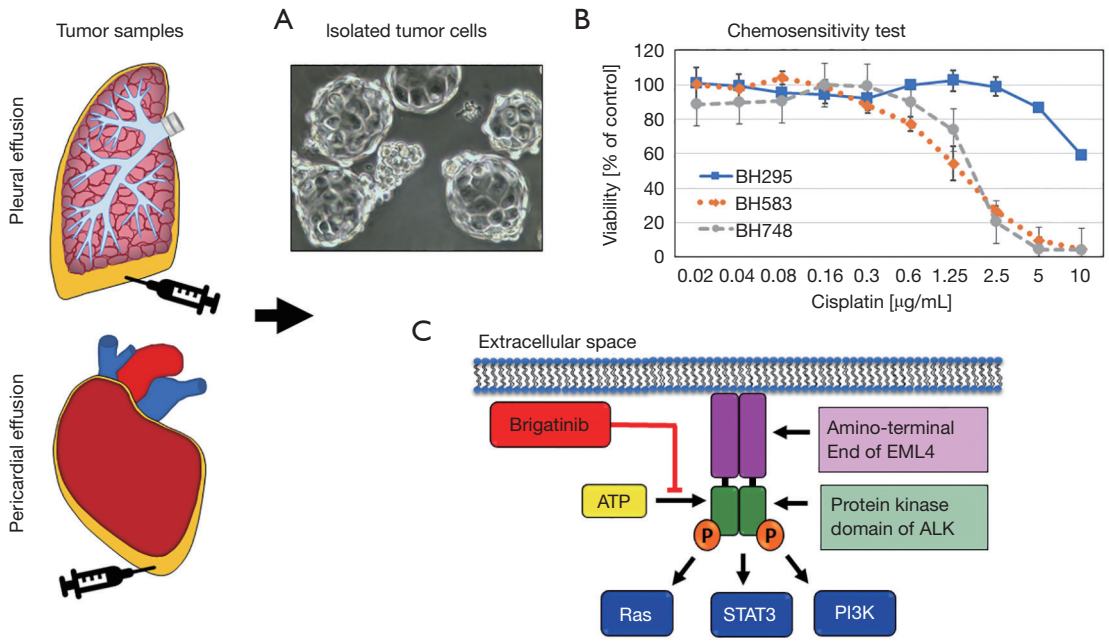

Figure 1 Scheme of drug testing of primary ALK-rearranged tumor cells. Tumor samples are collected from pleural or epicardial effusions and kept in tissue culture (A); ALK-rearranged tumor cells grow as hollow spheres. An example of a chemosensitivity test of 3 NSCLC cell lines is illustrated by dose-response curves for 10 twofold dilutions of cisplatin (B; mean values ± SD). The most frequent ALK rearrangement comprises the fusion of the kinase domain of ALK to EML4 domains resulting in constitutive activation of ALK (C) which triggers downstream RAS, STAT3 and PI3K pathways. ALK-directed TKIs, such as Brigatinib, block the kinase activity of the EML4-ALK fusion protein. ALK, anaplastic lymphoma kinase; NSCLC, non-small cell lung cancer.

respectively, which, furthermore, improves quality of life compared with chemotherapy. In the second line setting, crizotinib showed an ORR of 65% and 4 months of PFS-benefit in comparison with docetaxel or pemetrexed. Inevitably and like EGFR inhibition with EGFR TKIs, resistance to ALK inhibition develops in an average of 1 year (29). Only 30% of cases of acquired crizotinib resistance in patients with ALK-rearranged NSCLC stem from various secondary mutations of ALK, with the remaining 70% of such cases being due to other mechanisms and the vast majority of new or progressing lesions develop intracranially (30,31). After the failure of crizotinib, the second-line inhibitors ceritinib and alectinib have shown clinical responses. Additionally, the newer ALK TKIs generally provide more pronounced intracranial activity than crizotinib. The FDA granted accelerated approval of ceritinib in April 2014, for patients who progressed while receiving crizotinib. Alectinib received a similar approval for the same population in December 2015 followed by brigatinib in April 2017 (32).

Resistance to ALK inhibitors

Mechanisms of resistance to ALK TKIs are classified as either ALK-dependent "on-target" mechanism including secondary ALK mutations and amplifications or ALK-independent "off-target" mechanisms involving alternative signaling pathways and lineage transformations (33,34). In crizotinib resistance, secondary mutations occur in 20–30%, but for patients resistant to next-generation ALK-inhibitors, the frequency of ALK secondary resistance mutations increases to 50–70%. The occurrence of the resistant G1202R mutation is common and represents 21%, 29% and 43% of patients in cases resistant to ceritinib, alectinib and brigatinib, respectively (35). Lorlatinib, the third-generation ALK inhibitor has been shown to overcome resistance to this mutation (36). Alternatively, ALK-independent mechanisms comprise alterations in EGFR, KRAS, BRAF, MET, HER2 and KIT (37). Consequently, this prompted the development of newer generation ALK TKIs to overcome these resistance patterns, and these drugs include ceritinib, alectinib, brigatinib, ensartinib and

lorlatinib. Brigatinib has demonstrated a broad spectrum of preclinical activity against crizotinib-resistant ALK mutant NSCLC (38). Brigatinib acts as a multi-kinase inhibitor with a broad-spectrum activity against ALK, ROS1, FLT3, mutant variants of FLT3, IGFR-1R and T790M-mutant EGFR. Brigatinib displayed superior activity compared to crizotinib, ceritinib, and alectinib, against all 17 secondary ALK mutations, including *C1156Y*, *I1171S/T*, *V1180L*, *L1196M*, *L1152R/P*, *E1210K*, *G1269A* and the most refractory G1202R mutation (39). G1202R the only mutation so far associated with clinical resistance to all three previously approved ALK inhibitors.

Lorlatinib is the latest addition to the four targeted drugs currently available in the clinic via regular approval (5). Lorlatinib was specifically developed to cross the blood-brain barrier and to retain potency to acquired resistant mutations, including the ALK G1202R mutation. The frequency of this mutation increases significantly after treatment with second-generation agents (35). The presence of ALK resistance mutations is highly predictive for sensitivity to lorlatinib, whereas those cell lines without ALK mutations are resistant. Lorlatinib showed marked overall and intracranial activity both in treatment-naive patients and in those who had progressed on crizotinib, second-generation ALK inhibitors, or after up to three previous ALK inhibitors (40). Based on phase I and preliminary phase II data, lorlatinib received accelerated approval by the FDA in November 2018 for patients whose disease progressed on crizotinib or at least one other ALK inhibitor (alectinib or ceritinib). The ORR was 48%, with a complete response in 4% of patients and the estimated median duration of response 12.5 months. The intracranial ORR in 89 patients with measurable central nervous system (CNS) lesions was 60%, with a complete response in 21% and the estimated median duration of intracranial response was 19.5 months (40). A phase III study of lorlatinib vs crizotinib in first line treatment of patients with ALK-positive NSCLC is currently recruiting patients (NCT03052608).

A number of third-generation ALK inhibitors, such as entrectinib (RXDX-101) and ensartinib (X-396) is under trial and additional next-generation ALK inhibitors such as belizatinib (TSR-011), ASP3026, TPX-0005, F17752, CEP-37440, CEP- 28122, and GSK1838705A are under development (41). The new agents are expected to show enhanced anti-ALK activity, to improve the control of CNS disease, and to overcome or delay development of high-grade resistance mutations.

Sequence of the application of ALK inhibitors

The traditional approach to the treatment of patients with advanced-stage NSCLC harboring ALK rearrangements or EGFR mutations has been the sequential administration of therapies, in which patients first receive first-generation TKIs, which are eventually replaced by next-generation TKIs and/or chemotherapy upon disease progression, in a decision optionally guided by tumor molecular profiling (42). In the past few years, this strategy has been challenged by clinical evidence showing improved PFS, improved intracranial disease control and a generally favorable toxicity profile when next-generation EGFR and ALK TKIs are used in the first-line setting.

Experience with sequential treatment at our own center revealed that brigatinib as a second-line or later-line treatment in patients with ALK-rearranged NSCLC who developed resistance to crizotinib followed by various TKIs resulted in a disease control rate of 84.8% (43). Crizotinib was the single previous treatment for 15 (42.9%) of the patients, while crizotinib followed by ceritinib had been administered to 12 (34.3%) of the patients. Alectinib monotherapy had only been used for one patient, crizotinib followed by alectinib for two patients, crizotinib followed by ceritinib and alectinib for one patient, and ceritinib followed by alectinib for two patients (43). Seven of the 13 (53.8%) patients with brain metastases at baseline responded to brigatinib and the median PFS for the whole patient group was 9.9 months (range, 1–21 months). In all other subgroups (i.e., the various previous treatment combinations), all of the patients showed partial responses, with the exception of the two who had received ceritinib followed by alectinib which experienced disease progression under brigatinib.

The real problem is how to appropriately sequence therapy to obtain the most clinical benefit for the patients. The J-ALEX study introduced new insight into upfront treatment with second generation *ALK* inhibitors, demonstrating an improved PFS favoring alectinib (not estimable) over crizotinib (10.2 months) in the first line setting (HR 0.34). Brigatinib showed an ORR of 45% to 54% with a PFS of 9.2 to 12.9 months in the second-line setting (44). In conclusion, as distinct recommendations for subsequent therapies based on resistance mutation patterns are lacking, testing of these patterns will most likely not gain the same acceptance in clinical practice as for EGFR resistance mutation testing, at least not in the short term (43).

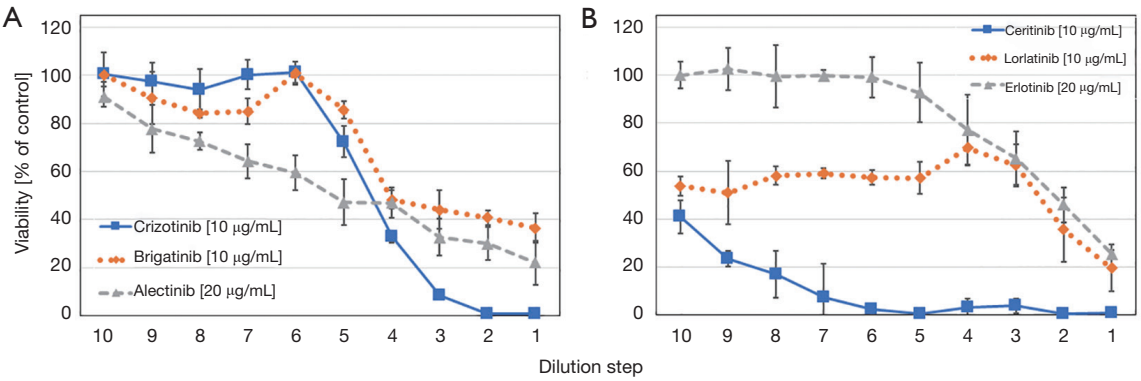

Figure 2 ALK-directed TKI chemosensitivity test of pleural tumor cells of a patient progressing under crizotinib and brigatinib. The tumor cells were exposed to 10 twofold dilutions of the indicated ALK-directed TKIs, starting at the concentrations given in (A) and (B), respectively. Resistance was observed for crizotinib, brigatinib and erlotinib, with partial resistance to alectinib and lorlatinib, and chemosensitivity to ceritinib. ALK, anaplastic lymphoma kinase; TKI, tyrosine kinase inhibitor.

ALK inhibitors and precision medicine

The progress of genomic profiling and NGS enables patient-centered targeted therapy, now termed precision cancer medicine (45). In NSCLC ALK-positive patients, the knowledge about primary and, particularly, secondary resistance mutations were expected to facilitate the selection of the most optimal treatment sequence. The US National Cancer Institute (NCI) is developing a "Master Protocol" for the selection of the treatment of patients with ALK-rearranged advanced NSCLC, in which the different mutations will guide therapy and drug sequence (46). Such a protocol is defined as a comprehensive operating procedure aiming at evaluating multiple hypotheses of sub-studies which are commonly conducted on cohorts based on specific tumor types, histologic subtypes, and/or molecular markers (47).

However, alterations of ALK fusion proteins are complex and comprise a number of distinct fusion partners and variants as well as a range of mutations providing distinct chemosensitivities and mechanisms of resistance to inhibitors. In genome-guided therapy, the relationship of drug sensitivity to various alterations of the target in a variable cellular environment may be difficult to establish. Actually, genomic-based cancer precision medicine has so far been successfully applied only for small minority of patients with cancer for which matching drugs have been available (48,49). The situation in ALK rearranged NSCLC is unique insofar as a number of alterations linked to chemoresistance is known and, in contrast to other cancers and various genetic alterations, a large collection of efficacious drugs is available.

A more direct correlation between the characteristics of individual tumors and their chemosensitivity is investigated in the frame of the so-called functional precision medicine. In detail, tumor cells of patients are isolated and exposed *in vitro* to a range of appropriate drugs to test their sensitivity and guide clinical therapy in conjunction with genomic data. ALK-rearranged tumors progressing under therapy tend to grow aggressively and a major fraction of the patients show accumulation of pleural or pericardial fluid containing significant numbers of tumor cells. These cells can be easily collected by aspiration and immediately used for *in vitro* assays (50). In case of ALK-positive NSCLC, the whole range of inhibitors can be applied to select the most effective sequential drug matching the individual patient's tumor. For these patients, rebiopsy or detection of ALK rearrangements in liquid biopsy is not necessary for selecting active agents (51).

An example of such an ALK TKI sensitivity assay is shown in *Figure 2*. Tumor cells are collected from pleural or pericardial effusions and transferred to tissue culture. ALK-rearranged cells are often assembled as hollow spheres and partially attached to the culture flasks (*Figure 2*). Typical experiments showing the chemosensitivity of three NSCLC lines to cisplatin and ALK inhibitors are presented in *Figures 1,2*. Half maximal inhibitory concentrations (IC_{50}) can be calculated from these dose-response curves and compared to the corresponding peak plasma concentrations to estimate the sensitivity of the tumor cells in the clinical

situation. In particular, the ALK-rearranged tumor cells can be treated with all kinds of the respective TKIs and the likeliness of clinical response to a specific agent concluded. Additionally, the *in vitro* sensitivity data may be linked to the genome-wide alterations detected by NGS. Obstacles of this kind of tests may be the lack of sufficient tumor cells, questionable validity of the primary pleural tumor cells as representatives of the original tumor and tumor cell heterogeneity. Such assays are under development and need to be clinically validated in larger patient cohorts. However, these tests could identify the most active inhibitors within a short time frame in part of the patients allowing for short-term decision making.

Conclusions

Compared to chemotherapy, the survival of ALK-rearranged NSCLC patients had been markedly improved by treatment with inhibitors directed to the tyrosine kinase moiety of rearranged ALK. Treatment of ALK-rearranged NSCLC is a success story of targeted cancer therapy. The first successful inhibitor crizotinib is now being replaced by second-line drugs such as alectinib, ceritinib and brigatinib which show also improved activity as first-line agents. Most importantly, the newer ALK inhibitors are distinguished by high activity against brain metastases, a frequent site of secondary lesions in these patients. Development of resistance to all these drugs can be overcome in most patients by the third-line drug lorlatinib, although half of the patients which are refractory to treatment seem to have various mechanisms of resistance different from ALK mutations. Unfortunately, the handling of resistance mediated by ALK mutations is not as straightforward as in case of the progressive alterations of the EGFR in NSCLC. Currently, the optimal sequence of the administration of the ALK inhibitors or combinations thereof or with other therapeutics is not clear. Data on ALK rearrangement variants or mutations may be used to select the appropriate therapy. In the future, functional genomics studying the *in vitro* chemosensitivity of pleura-derived tumor cells may match individual tumors with effective drugs in suitable patients.

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Footnote

Conflict of Interest: The authors have no conflicts of interest to declare.

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