Review Article

# Advance of theragnosis biomarkers in lung cancer: from clinical to molecular pathology and biology

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**Abstract:** One distinct molecular subtype of non-small cell lung cancer (NSCLC) is defined by rearrangement of the anaplastic lymphoma kinase (*ALK*). The increasing knowledge over the last years has enabled the continuous improvement of ALK inhibitors; however, resistance in these patients remains a major concern. In this review, we summarize recent findings in *ALK*+-adenocarcinoma of the lung, highlighting the role of *TP53* mutations in this specific cancer type and suggest new diagnostic strategies for the future, in order to improve patient's outcome.

**Keywords:** Non-small cell lung cancer (NSCLC); anaplastic lymphoma kinase (*ALK*); *ALK*+-adenocarcinoma; *TP53*; biomarker

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Lung cancer is the leading cause of cancer deaths in men and women worldwide (1). Historically, it was divided in two major groups—small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) with 80% to 85% of cases belonging to the latter group (2). Over the last years, however, the advent of next-generation sequencing (NGS) technologies and sequencing studies revealed that this classification is outdated and that particularly the term "non-small cell lung cancer" involves an extremely heterogeneous set of diseases at the molecular level that translates into both, tumors' clinical behavior and therapeutic decision-making (3).

A specific molecular subtype of NSCLC is defined by rearrangements of the anaplastic lymphoma kinase (*ALK*), which is found in about 4% of patients with lung adenocarcinomas (4) predominantly at younger age and mostly in light- or never-smokers (5). Histologically, it is often, but not exclusively, characterized by poor-differentiation with acinar-predominant structure and a mucin/signet-ring cell pattern (6,7).

The *ALK* gene encodes a receptor tyrosine kinase, which plays a significant role in the development and function of the nervous system, where it controls the basic mechanisms of cell proliferation, survival, and differentiation in response to extracellular stimuli (8). Its activation results in dimerization and autophosphorylation of the kinase domain consequently activating downstream signaling pathways, such as the RAS/MAPK, PI3K/AKT, and JAK/STAT pathways (9). ALK is considered an orphan receptor with unknown ligands, however, several factors have been described to interact with the receptor and activate ALK downstream signaling (10-13). Under physiological conditions, ALK is only embryonically expressed in early developmental regulation but not in the adult lung.

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Under pathologic conditions, such as *ALK* translocated-NSCLC, the *ALK* gene brakes and fuses with diverse partners, leading to a constitutively activated kinase resulting in the uncontrolled activation of its downstream signaling pathways. These signaling cascades can contribute to uncontrolled cell proliferation, survival migration, angiogenesis and metastasis (14).

*ALK* fusions arise from breakage of the *ALK* gene, located on chromosome 2, and subsequent fusion of the 3' end of *ALK*, and the 5' portion of a different gene providing its promoter. The *ALK* breakpoint lies most frequently within intron 19 and, rarely, within exon 20, preserving the tyrosine kinase domain. The most common fusion partner is *EML4* (echinoderm microtubule-associated protein-like 4), however, multiple different 5' translocation partners have been identified, including *KIF5B*, *KLC1*, *TFG*, *TPR*, *HIP1*, *STRN*, *DCTN1*, *SQSTM1*, *BIRC6* (15). *EML4* and *ALK* each map to the short arm of chromosome 2 but have opposite orientations, due to inversion of *EML4* (16). The precise underlying mechanisms triggering the development of *ALK* gene rearrangements are not fully understood yet. In contrast to *ALK*, the *EML4* breakpoints differ frequently, defining different variants of the *EML4-ALK* fusion gene. To date, more than 15 *EML4-ALK* variants have been identified with some variants being expressed as multiple isoforms, as reviewed in (17). All variants share the intracellular kinase domain of ALK, and contain the trimerization domain of EML4, which is required for the constitutive activation of ALK through oligomerization and autophosphorylation (18).

For the detection of *ALK* rearrangements in the routine clinical setting most laboratories use IHC (immunohistochemistry) and/or FISH (fluorescence *in situ* hybridization), which is cost effective and easily applicable with the disadvantage that other fusion partners than *EML4* cannot be determined. However, molecular approaches, such as RNA-sequencing analyses by Archer®FusionPlex® have recently emerged as relevant alternatives and may provide an effective and accurate alternative to FISH testing for the detection of both, known and novel *ALK* rearrangements in clinical diagnostic settings (19).

In 2010, Kwak *et al.* were the first to show that the inhibition of ALK in *ALK*+-lung tumors by the multi-targeted tyrosine kinase inhibitor (TKI) crizotinib resulted in tumor shrinkage or stable disease in most patients (20), and only one year later, crizotinib was approved by the FDA as the first licensed ALK inhibitor for *ALK*+-NSCLC (21). Despite the enormous efficacy of crizotinib, resistance continued to be a major impediment.

Due to recent analyses of post-treatment tumor tissue samples by Camidge *et al.* (22) our knowledge in terms of molecular resistance mechanisms to ALK inhibitors has been significantly extended (22). The authors defined two types of ALK-inhibitor resistance: the ALK-dominant type (also known as "on-target" type) represents 50% of cases and is defined by secondary mutations within the ALK kinase domain or gene amplifications of the *ALK* gene. The non-dominant type is characterized by the activation of ALK-bypassing oncogenic pathways, including *EGFR* and *KRAS* mutations (23), amplification of *KIT* (24) or activation of the IGF1R pathway (25). Further described resistance mechanisms are epithelial-mesenchymal transition (EMT) (26) and autophagy (27).

With increasing knowledge over the last years, therapeutic options for advanced *ALK*+-NSCLC have been continuously improved and increasingly potent and selective ALK inhibitors (e.g., ceritinib and alectinib) have been approved by the FDA (14). A recent phase III trial revealed that the second-generation ALK inhibitor alectinib showed superior efficacy and lower toxicity in primary treatment of *ALK*-positive NSCLC and was further associated with activity against CNS (28). Consequently, it outperformed the first approved ALK inhibitor crizotinib and has recently been used as first line therapy. Further next-generation inhibitors, such as brigatinib and lorlatinib are currently under development (29,30). Of note, in the past few years, the "traditional" strategy of sequential treatment approaches, in which *ALK*+-patients initially received first-generation TKIs, which were replaced by next-generation TKIs and/or chemotherapy upon disease progression, has been challenged and tended to shift to the use of next-generation TKIs in the frontline setting (31).

Despite the well-advanced elucidation of resistance mechanisms, it remains unclear why some patients relapse faster or show worse up-front response to ALK inhibition treatment. Recent data by Lin *et al.* showed that specific *EML4-ALK* variants may be associated with the development of resistance mutations to ALK TKIs in *ALK*+-NSCLC (32). In 2016, Gainor *et al.* performed the largest series of repeat biopsies (with 103 cases being studied) from patients with TKI-resistant *ALK*+-NSCLC, using a combination of genetic sequencing, histologic analyses, and functional drug screens (33). They found that each ALK inhibitor was associated with a distinct spectrum of ALK resistance mutations and that the frequency of one mutation—ALK G1202R—increased

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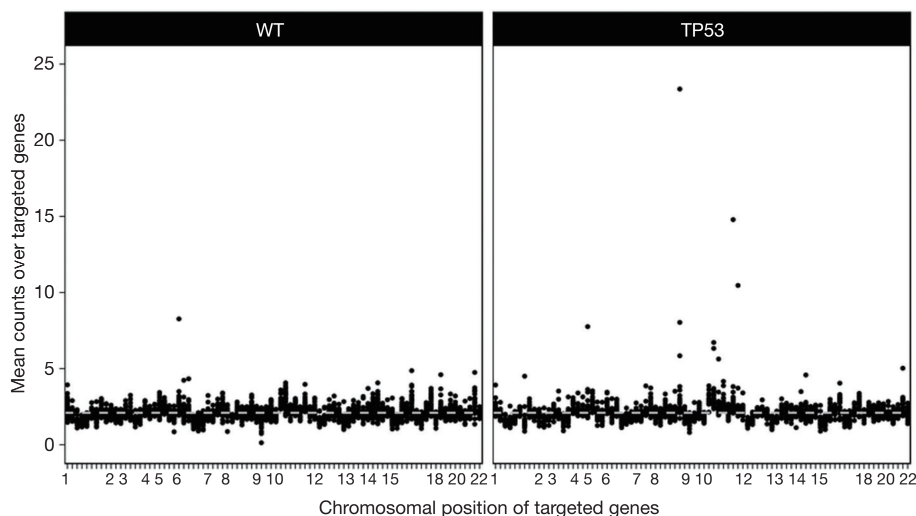


**Figure 1** Genomic instability in *ALK*+-adenocarcinomas of the lung with *TP53*-deficiency. Copy number plots of *ALK*+-lung adenocarcinoma cases from 31 patients without (A) and 21 patients with (B) *TP53* mutation. NanoString nCounter® technology was used to determine the copy number alterations of 87 cancer-relevant genes. Absolute copy numbers (Y-axis) for each gene are plotted according to their chromosomal location (X-axis). *ALK*, anaplastic lymphoma kinase; WT, wild-type.

significantly after treatment with second-generation agents. Interestingly, they revealed that 33% of *ALK*+-tumors exhibited mutations within *TP53*, but it remained unclear whether these mutations were present prior therapy or occurred in the course of TKI-treatment. One year later, it was found by Aisner *et al.* that concurrent *TP53* mutations are associated with a reduced survival in these patients (34), suggesting that molecular testing should be performed on all individuals with lung adenocarcinomas irrespective of clinical characteristics, in order to enable the detection of both targetable driver alterations and additional genetic alterations that have potential significance for therapy selection and as predictive markers for the efficacy of treatment. The authors assumed that *TP53* mutations result in genetic instability and thereby trigger the development of multiple resistance mechanisms to targeted therapy reducing the survival rate in *ALK*+-patients. Just a few months later, this assumption could be confirmed by our group, by analyzing gene copy number alterations of 87 cancer-relevant genes in *ALK*+-tumors and cell lines harboring either wild-type or mutated *TP53* with NanoString nCounter® technology (35). We found that *TP53* mutations occurred in the early phase of tumorigenesis and that these mutations could lead to chromosomal instability. Our analyses further revealed that 24% of *TP53*-mutated patients had amplifications of the following cancer genes: *MYC* (14%), *CCND1* (10%), *TERT* (5%), *BIRC2* (5%), *ORAOV1* (5%), *YAP1* (5%). A summary of these findings is depicted in *Figure 1*.

By ChIP-Seq analyses we further found MYC-binding sites within the promoter region of *EML4* in *ALK*+/*TP53*-mutated cells and *MYC*-overexpression resulted in elevated expression levels of the EML4-ALK protein, as well as increased cell proliferation rates, assuming a potential MYC-dependent resistance mechanism in patients with increased *MYC* copy numbers. In line with that and the aforementioned data by Aisner *et al.* (34), clinical data subsequently published by our group, confirmed that among 216 analyzed patients with *ALK*-rearranged NSCLC, the frequency of pathogenic *TP53* mutations was 23.8%, while other co-occurring mutations were rare events in pre-treatment biopsies (36). It was further shown, that these patients had a significantly worse median progression-free survival (PFS) and overall survival (OS) compared to *TP53* wild-type patients treated with either chemotherapy, crizotinib only or crizotinib followed by next-generation ALK-inhibitors [PFS 3.9 months (95% CI: 2.4–5.6) *vs.* 10.3 months (95% CI: 8.6–12.0), P<0.001; OS 15.0 months (95% CI: 5.0–24.9) *vs.* 50.0 months (95% CI: 22.9–77.1), P=0.002]. This difference was confirmed in all treatment-related subgroups, concluding that in *ALK*-rearranged NSCLC co-occurring *TP53* mutations predict an unfavorable outcome of systemic therapies.

Taken together, these new findings show that the

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subgroup of *ALK*+-adenocarcinomas of the lung is more heterogeneous than historically assumed and will potentially have implications for stratification in future clinical trials for *ALK*+-patients. However, these studies are limited, as most lung cancer patients are diagnosed at advanced disease, when surgical resection is not possible. Therefore, diagnoses are performed on small biopsies, impeding comprehensive genomic analyses. It is of great importance to elucidate these genomic differences in more detail in the future in order to better understand the patient's response to treatment and thereby improve their outcome.

The emergence of immunotherapy approaches, including monoclonal antibodies directed against cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) and programmed cell death protein-1 (PD-1) and its ligand (PD-L1) has redefined the management of lung cancer, achieving significant long-lasting responses with manageable safety profile. Current efforts are focusing on new potential combination strategies, using immune checkpoint blockade as a partner for targeted agents. However, there are few data available on the combination of checkpoint inhibitors with ALK TKIs in advanced NSCLC, and clinical trials are mostly still ongoing. Generally, it has been reported that patients with lung cancer caused by mutations or rearrangements within driver genes, such as *ALK*, do not show high response rates to immunotherapy in combination with checkpoint inhibitors as comprehensively reviewed in (37). In line with that, preclinical data on the treatment naïve *ALK*+-cell line H3122, which was administered to a combination of PD-1 and ALK inhibitor, did not show any synergistic tumor killing effects (38). It is known that tumor mutational burden (TMB), meaning the number of mutations carried by tumor cells, has an impact on tumor immunogenicity (39). TMB is reduced in lung cancers harboring *ALK* fusions as known drivers (40), which could explain the poor response to immunotherapy. A recent cohort study of 13 patients with advanced *ALK*+-NSCLC treated with a combination of nivolumab, an immune checkpoint inhibitor antibody which blocks PD-1, and crizotinib did not meet the primary endpoint of safety and tolerability for the first-line treatment, concluding that the findings do not support further evaluation of nivolumab and crizotinib in combination (41). In contrast, it was recently reported that the combination of full dose alectinib and atezolizumab in treatment-naïve *ALK*+-patients appeared to have an acceptable safety profile with no new safety findings for either agent. Despite the encouraging early efficacy results, further follow-up data are needed to define the benefit of this combination therapy (42). Retrospectively, it has to be noted that these patients were not stratified according to co-occurring mutations within other genes, such as *TP53*, which, as indicated by our own data, might be of great importance in future clinical settings.

Over the last years, our knowledge on *ALK*+-NSCLC has improved and revealed that the group of *ALK*+-NSCLC is more heterogeneous than initially thought. These new findings encourage intensive future research in order to better elucidate and understand the underlying molecular mechanisms occurring in drug resistance. This will lead to the development of new and more durable treatment strategies, which are even more personalized and consequently improve treatment outcome in patients.

Taken together, based on the current literature and our own findings, we suggest that an up-front assessment of *TP53* status in all *ALK*+-patients should be implemented in the routine clinical work-up. A rapid and cost-effective pre-screening could be performed by means of p53 IHC, knowing that *TP53* missense mutations result in a non-functional protein accumulation in the tumor cell nuclei, whereas truncating mutations result in the complete loss of p53. "Positive" cases should undergo additional sequencing analyses including assessment of copy number variations. In addition, it might be of importance to extend tumor analyses and include the determination of fusion partners and variants by using RNA-sequencing methods, such as Archer®FusionPlex® in the future to fully capture the genomic complexity of this group of genetically diverse tumors.

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## Footnote

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