

Identification of Somatic Genomic Alterations in Circulating Tumors Cells: Another Step Forward in Non–Small-Cell Lung Cancer?

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The last decade has witnessed substantial progress in defining the molecular determinants of non–small-cell lung cancer (NSCLC) and demonstrating how these can be exploited in the clinic. In 2004, the identification of somatic mutations in the epidermal growth factor receptor (*EGFR*) gene provided the first insight into a clinically relevant NSCLC driver oncogene.¹⁻³ *EGFR* mutations in NSCLC are transforming, enhance the activity of the kinase domain of *EGFR*, and increase the affinity for *EGFR* tyrosine kinase inhibitors (TKIs).⁴ In *EGFR*-mutated NSCLCs (ie, tumors with classic *EGFR* exon 19 deletions or the L858R mutation, which are found in approximately 15% of all NSCLCs), clinical and radiographic responses are achieved in most patients with the use of two commercially available reversible *EGFR* TKIs: gefitinib and erlotinib.⁵ These *EGFR* TKIs improve outcomes when compared with cytotoxic chemotherapies,⁶⁻⁸ and the evidence-based use of these drugs, as recommended by the American Society of Clinical Oncology⁹ and other practice¹⁰ and regulatory agencies, is now restricted to *EGFR*-mutated NSCLCs in the first-line treatment of advanced tumors.

The significant palliative benefits that are offered by matching a tumor driver mutation with an appropriate inhibitor paved the way for the genomic characterization of NSCLCs and the development of novel TKIs that can target these changes. The deciphering of NSCLCs using next-generation sequencing (through whole-genome, exome, and transcriptome techniques) has identified numerous driver oncogenic events (ie, activating mutations or rearrangements) involving targetable kinases, including *ROS1*, *ERBB2*, *BRAF*, and *RET*, among others.¹¹⁻¹⁴ The separation of NSCLCs into distinct, actionable subtypes is mostly clear in lung adenocarcinomas of never-smokers (Figure 1), in which almost all tumors have a mutually exclusive driver oncogene for which TKIs are either clinically available or in early- to late-stage development.

Amid the exciting series of discoveries that have occurred during the last decade, one of the most remarkable has been the story of how anaplastic lymphoma kinase (*ALK*) gene alterations became a biomarker and therapeutic target in NSCLC. *ALK* is a transmembrane tyrosine kinase that had been previously implicated in the pathogenesis of anaplastic large cell lymphoma and neuroblastoma.^{15,16} In 2007, a Japanese group from Jichi Medical University identified novel fusion oncogenes involving the kinase domain of *ALK* in patients with

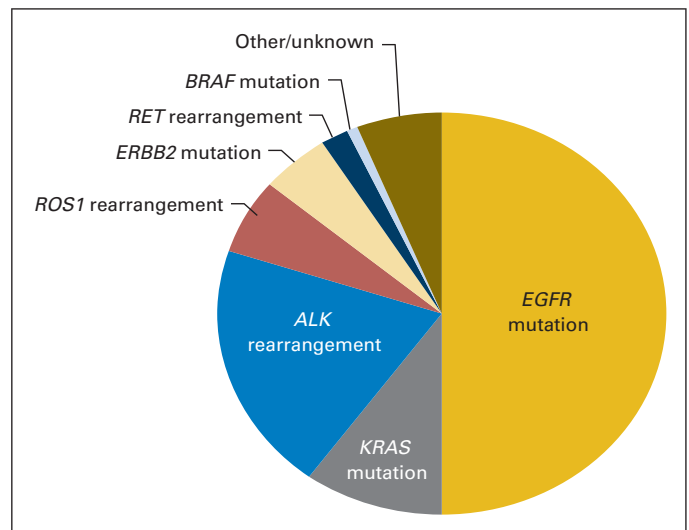


Fig 1. Pie chart of frequent somatic driver mutations in never-smokers with lung adenocarcinoma. The frequencies described are approximations adapted from other sources.¹¹⁻¹⁴

NSCLC.¹⁵ *ALK* rearrangements, either inversions or translocations, characterize the genomic changes observed in NSCLC.¹⁶ The most frequent event is an inversion in the short arm of chromosome 2 that results in the fusion of the echinoderm microtubule-associated protein-like 4 (*EML4*) with *ALK*, leading to the production of an *EML4-ALK* fusion tyrosine kinase.^{15,17} Several *EML4-ALK* variants have now been described, with *EML4-ALK* E13;A20 and E20;A20 being the most common.¹⁸ Few other tumor types harbor these *EML4-ALK* rearrangements, and it remains unclear why NSCLCs can acquire these somatic changes. Interestingly, some clinical and pathologic features are associated with an increased incidence of tumors containing *ALK* rearrangements. These include never or light smoking history (< 15 pack-years), young age, and adenocarcinoma histology with signet rings.¹⁹⁻²³ The reported prevalence of *ALK* rearrangements in NSCLC is approximately 5%¹⁶; however, in never-smokers or light smokers with lung adenocarcinoma, the prevalence may be as high as 20%.²² Therefore, as many as 10,000 new cases of *ALK*-rearranged NSCLC are expected in the United States this year.²⁴

EML4 and other less frequent fusion partners, such as TRK-fused gene and kinesin family member 5B, participate in ligand-independent dimerization and activate the ALK tyrosine kinase domain,^{25,26} leading to oncogenic fusion kinases that trigger the mitogen-activated protein kinase–extracellular regulated kinase as well as phosphatidylinositol 3-kinase–AKT pathways.^{15,27} The dependency of *ALK*-rearranged NSCLC on ALK-mediated signals quickly translated into successful drug development in the clinic, at a record pace.²⁸

ALK inhibitors—in particular, ALK TKIs—initially were shown to have activity against *ALK* rearrangements in preclinical models.^{21,29–32} The most advanced TKI targeting *ALK* rearrangements in NSCLC is called crizotinib (formerly PF02341066); it was developed as an inhibitor of MET but also functions as a multitargeted TKI against ALK^{29–31} and ROS1.^{12,33} As a result of significant contributions and collaborations between academic medical centers,¹⁶ the sponsoring pharmaceutical company,³⁴ and patient advocates,³⁵ crizotinib fast-tracked its way through the clinical development phase.²⁸ The outcomes of *ALK*-rearranged NSCLCs that were included in the original phase I trial of crizotinib led to an expansion cohort,³⁴ and subsequently, proper phase II (PROFILE1005 [An Investigational Drug, PF-02341066, Is Being Studied in Patients With Advanced Non-Small Cell Lung Cancer With a Specific Gene Profile Involving the Anaplastic Lymphoma Kinase Gene]) and III registration trials (PROFILE1007 [An Investigational Drug, PF-02341066, Is Being Studied Versus Standard of Care in Patients With Advanced Non-Small Cell Lung Cancer With a Specific Gene Profile Involving the Anaplastic Lymphoma Kinase Gene], and PROFILE1014 [A Clinical Trial Testing the Efficacy of Crizotinib Versus Standard Chemotherapy Pemetrexed Plus Cisplatin or Carboplatin in Patients With ALK Positive Non Squamous Cancer of the Lung]). Crizotinib 250 mg orally twice per day was chosen as the initial recommended dose, and the early clinical and radiographic results were quite remarkable.³⁴ Between 2008 and 2011, more than 149 patients with *ALK*-rearranged NSCLC were enrolled onto the phase I trial; 60.8% had radiographic responses with a median progression-free survival of 9.7 months and an estimated overall survival of 74.8% at 1 year.³⁶ Nearly identical results were observed in the larger phase II trial (PROFILE1005) of crizotinib.^{16,28} These combined results led to US Food and Drug Administration approval of crizotinib on August 26, 2011, for metastatic NSCLC that is positive for *ALK* rearrangements.^{16,28,34} The recent results of the randomized PROFILE1007 trial have unequivocally shown that crizotinib leads to improved outcomes (response rate, progression-free survival [median 7.7 v 3.0 months; hazard ratio, 0.49; 95% CI, 0.37 to 0.64; $P < .001$], and quality-of-life measurements) when compared with standard cytotoxic second-line chemotherapies (ie, docetaxel or pemetrexed) in *ALK*-rearranged NSCLC.³⁷

The approval of crizotinib was linked to the approval of a test to identify *ALK* rearrangements.^{16,34,38} The test selected for use in clinical trials and routine clinical specimens was the Vysis ALK Break Apart fluorescent in situ hybridization (FISH) probe (Abbott Molecular, Des Plaines, IL), which also received US Food and Drug Administration approval in 2011.³⁸ The 5' and 3' fluorescent probes bind to areas upstream and downstream of the rearrangement breakpoint in exon 20 of *ALK*.³⁸ A patient's tumor is generally defined as being ALK positive if more than 15% of cells show a rearrangement involving *ALK* indicated by split of the 3' and 5' probes or by an isolated 3' signal.^{16,38} As of now, *ALK* FISH requires tissue acquisition in the form of a biopsy, aspirate, or cell block preparation. The need for adequate

tissue has its limitation in clinical practice, specifically in advanced NSCLC, for which small quantities of tissue are used for multiple procedures (histologic staining, diagnostic immunohistochemistry, and multiple molecular analyses).

In the article that accompanies this editorial,³⁹ a group from the Institut de Cancérologie Gustave Roussy attempted to avoid the need for a tissue sample to diagnose *ALK*-rearranged NSCLC by studying a novel *ALK* FISH method in circulating tumor cells (CTCs). CTCs were enriched on the basis of blood filtration using isolation by size of epithelial tumor cells. The main technologic advance in using the US Food and Drug Administration–approved Vysis ALK Break Apart FISH probe in CTCs was the development of a filter-adapted FISH (FA-FISH) assay. The FA-FISH consists of a filter to capture CTCs enriched by blood filtration, followed by preparation of the filter spot to fixate cells, and finally the analysis of cells using the aforementioned FISH assay.³⁹ Using an alternative cutoff value of more than four *ALK*-rearranged CTCs per 1 mL—instead of the 15% cell positivity threshold in tissue—the authors reported an exceedingly high 100% sensitivity and specificity of their FA-FISH method when compared with *ALK* FISH on paraffin-embedded tumor samples.³⁹ The FA-FISH assay was also successfully used to monitor quantitative and qualitative changes of CTCs from *ALK*-rearranged NSCLCs while patients received crizotinib.³⁹ The *ALK*-rearranged CTCs that were identified very interestingly disclosed a mesenchymal phenotype, which may indicate that the CTCs from these tumors are unique in their invasiveness and migratory pattern when compared with the heterogeneous tissue tumor bulk.³⁹ Another French group had previously reported the ability to use *ALK* FISH to detect *ALK* rearrangements in CTCs.⁴⁰ These are the first examples of how an *ALK* FISH probe can be used in CTCs.

Despite the attractiveness and potential convenience of using blood-based assays (including CTCs) to diagnose genomic alterations and follow response to therapy (ie, as a liquid biopsy) in NSCLCs, these technologies have been fraught with significant hurdles^{41–43} and have not gathered momentum to supplement tissue-based diagnostics. The main issues with the use of CTCs are the lack of a standardized method to define and capture these cells^{42,43} and the technical challenges in capturing the one CTC among the other billion circulating blood cells.⁴¹ The only US Food and Drug Administration–approved, commercially available CTC detection system is the CellSearch CTC test (Veridex, Raritan, NJ), which enriches CTCs by using particles that are coated with antibodies against the epithelial cell adhesion molecule (EpCAM)⁴¹ and is approved as a prognostic test in breast, colon, and prostate cancers. However, it is not currently approved for a lung cancer indication because of lack of confirmatory trials that indicate its usefulness as a clinically significant prognostic or predictive marker in NSCLC.^{41,43} Other enrichment methods, such as isolation by size of epithelial tumor cells, can be used to identify CTCs that have lost the expression of epithelial markers,^{41–43} but again lack sufficient clinical development to achieve regulatory approval. Even if these rare CTCs could be routinely identified, captured, and isolated using available technologies, the other main limitation of their use for genomic studies is the minute amounts of DNA, RNA, and protein that can be collected from CTCs that are derived from standard volumes of whole blood samples.^{42,43} Despite these obvious limitations, some attempts to use CTCs for genomic characterization of driver oncogenes in NSCLC have been successfully reported.⁴³ In 2008, it was reported that *EGFR* mutation status could be assessed from peripheral

blood samples by examining captured CTCs with sensitive allele-specific genotyping techniques.⁴⁴ The results showed that genomic DNA extraction was feasible and that the expected mutation was identified in most cases of *EGFR*-mutated NSCLC during therapy with *EGFR* TKIs.^{41,43,44} Other blood-based assays, using tumor-shed DNA that has been isolated from serum or plasma, have also been partially successful in identifying single driver mutations in patients with advanced NSCLC.⁴⁵

The nascent field of single gene characterization of CTCs lags significantly behind the exponential curve of genomic events that are part of clinical practice and clinical trial design in NSCLC.⁴⁶ As an example, all cases of advanced adenocarcinoma of the lung are expected to be tested for—at a minimum—*EGFR* mutations and *ALK* rearrangements, which require the diagnostic tissue to be procured for DNA extraction and prepared for a FISH assay, respectively. As new driver oncogenic events are proposed as potential biomarkers, an increasing number of single tests (either more DNA- or RNA-based tests, or more FISH assays) will have to be added to the expanding list of clinically useful assays of predictive markers in this disease. The US Food and Drug Administration's important proposal to develop companion diagnostic devices/tests simultaneously with biomarker-based drug evaluation might further increase the complexities of testing requirements.⁴⁷ Potential ways to mitigate this situation include bundling genomic tests that use the same starting material (ie, use of multiplexing genotyping techniques when DNA is isolated) or using targeted next-generation sequencing to obtain somatic mutational events, rearrangements, and copy-number changes in tissue-derived specimens.⁴⁸ The latter efforts seem to be gaining momentum as the cost of whole genome sequencing decreases and the robustness of the technology increases. It is foreseeable to envision that most—if not all—new cases of NSCLC will be able to have nearly complete genomic characterization by the end of this decade using one unified testing protocol that will not require excessive quantities of tumor-derived material. Once the field achieves this goal, the next logical step will be to use these technologies to diagnose patients, select biomarker-based antineoplastics, and monitor response to therapies in NSCLC, using not only pathologic tissues but also CTCs and/or other blood-based assays.

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