The article by Rodig and colleagues, which was published in the August 15, 2009, issue of Clinical Cancer Research, helped detail the clinicopathologic profile of ALK-positive tumors and initiated the development of ALK diagnostics. Subsequent clinical trials utilized these findings, resulting in the approval of effective targeted therapies. The ALK story is a model for the development of therapies in the genomic era. Clin Cancer Res 21(23): 5185–7. ©2015 AACR. See related article by Rodig et al., Clin Cancer Res 2009;15(16) August 15, 2009;5216–23

It has been 8 years since the description of EML4–ALK gene fusions by Dr. Hiroyuki Mano’s laboratory in 2007, and 6 years since our 2009 publication in this journal of the article by Rodig and colleagues, “Unique Clinicopathologic Features Characterize ALK-Rearranged Lung Adenocarcinoma in the Western Population” (1, 2). Our study attempted to build on Dr. Mano’s seminal discovery by performing a careful analysis of the clinical and pathologic features of patients with ALK fusions. In subsequent years, we have seen major clinical breakthroughs in the ALK fusion–positive lung cancer population, with approval of three small-molecule inhibitors (3–6). These drugs have extended the lives of many ALK-positive lung cancer patients, and with advances in our understanding of drug resistance, we should see continued survival improvements.

It was clear at the time from the original description of EML4–ALK fusions that the ALK kinase domain would be an important drug target. However, it was unclear how common ALK fusions would be in a larger cohort, and the prevalence was completely unknown in the Western population. For drug development to proceed efficiently, knowing the prevalence data was critical. Also, detailed understanding of the clinical parameters of the ALK population was lacking, including any correlation with age, sex, stage, and histologic subtype. Finally, the optimal diagnostic modality to move ALK screening into the clinic was unclear, with PCR, FISH, and immunohistochemistry (IHC) all being possible options.

To initiate the study we retrieved a total of 358 lung adenocarcinoma samples of diverse stages, including 116 surgically resected lung cancers from Brigham and Women’s Hospital, 111 surgically resected samples from the University of Pittsburgh, and 131 advanced-stage lung cancer samples from the Massachusetts General Hospital (MGH). The clinical characteristics were retrieved, hematoxylin and eosin slides were reviewed using the then accepted World Health Organization histologic classification, and both FISH and IHC were performed on unstained slides. As with most such studies the hard work was in the organization and careful annotation of clinical parameters.

It quickly became apparent that ALK-positive tumors in the Western population presented at a high stage. Although we found an overall study prevalence of ALK fusions of 6%, in the MGH advanced-stage cohort, we found 19 of 131 (14%) ALK-positive cases, with the majority limited to stage IV disease. This result alone was an important finding and suggested that ALK-positive tumors would have an aggressive natural history. The 14% prevalence in the MGH population, in retrospect, was an inflated number even within stage IV disease. It is now generally accepted that ALK-positive tumors comprise 3% to 5% of lung adenocarcinomas. This inflation was due, almost certainly, to case ascertainment bias. MGH, as a result of the groundbreaking work of Drs. Thomas Lynch and Daniel Haber on EGFR, was a major referral center for nonsmoking lung cancer patients. A second highly significant finding in our study was that ALK fusions were clearly more common in nonsmokers, explaining the high prevalence number. It should be clarified that ALK fusions (and EGFR mutations and ROS1 fusions) are not limited to nonsmokers (that would suggest that smoking was protective against developing those alterations), but arise independently of smoking. Thus, the percentage of smokers in the ALK population should be the same as the percentage of smokers in the general population. A very important conclusion is that ALK clinical screening should be performed on all patients with lung adenocarcinoma, and not just nonsmokers. Regulatory agencies and insurance payors have debated this topic and in some cases misinterpreted the association with nonsmoking, and in the case of Palmetto have decided only to perform on all patients with lung adenocarcinoma.

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We also noted that the ALK-positive patients were younger than the ALK-negative population by an average of more than 10 years. ALK fusions were mutually exclusive from EGFR mutations in our cohort. A final statistically significant finding was that the ALK-positive samples commonly had a solid growth pattern, had an overrepresentation of cells producing mucin, and often had signet ring cells. Although signet ring cell differentiation was well...
described in lung cancer, there was no real genetic correlate at that point. All together, we described a very clear clinicopathologic profile of ALK-positive patients: the prototype was a young, nonsmoking patient with an advanced-stage adenocarcinoma with solid and signet ring cell morphology.

Finally, we attempted to begin the analysis of diagnostic methods that would be optimal for ALK screening in the clinic. The method would need to be performed on formalin-fixed paraffin-embedded archived material and should of course have excellent performance characteristics. The limitations of ALK FISH are now well known and include the need for FISH expertise, which is not always present in pathology laboratories, and the particular difficulty in interpreting the EML4-ALK chromosome 2 inversion that results in a very subtle split. In our hands, however, ALK FISH performed well as we gained more experience and has become the gold-standard diagnostic assay in the United States. IHC has many advantages, including ease of use and cost, but at the time commercial ALK antibodies still required optimization. IHC would need several years to catch up to FISH. Today both assays are very sensitive and have been validated in large clinical trials. We now are moving into the era of genomics, and many labs, including our own, have moved to next-generation sequencing (NGS; ref. 8). Sequencing offers the benefit of direct identification of the ALK fusion partner and the exact sequences at the fusion breakpoint.

What impact did our findings have? We hoped that our work would help identify ALK-positive patients, a relatively rare genetic subtype, for enrollment in the early-phase crizotinib trials based on clinical parameters. The fact that the trials progressed so fast with brisk accrual is in part due to the clear clinical presentation that allowed identification of likely ALK-positive cases even before ALK genotyping was standard of care. Now oncologists could find patients that met the above characteristics and refer them for testing, and patients themselves could self-identify, advancing trials significantly, and in the end accelerating the approval of crizotinib and later ceritinib and alectinib (3–5).

Research published subsequent to our study has clearly corroborated our findings, including the correlation of ALK fusions with young age, nonsmoking status, advanced stage, mutual exclusivity from other major driver genes, and signet ring cell morphology (9–13). Recently several meta-analyses have been published that have firmly established these findings, although some minor differences have been noted in the morphology of ALK-positive tumors in Asia (14).

Soon after this study was completed, we began analysis of another gene fusion event in lung cancer involving the ROS1 gene (15). ROS1 fusions in lung cancer were described soon after ALK fusions (16), and we managed to utilize many of the same samples to perform a parallel analysis of the ROS1-positive population. We quickly realized that ROS1-positive patients had nearly identical clinical features as the ALK-positive patients, apart from signet ring cells being a rare finding. The ALK studies allowed us to move very quickly in this analysis and defined a growing list of targetable drivers in lung cancer. Fortuitously, ROS1 is structurally related to ALK to the extent that it is effectively inhibited by crizotinib. Our group has recently confirmed the effectiveness of crizotinib efficacy in an expanded phase I study (17). Although there is a growing list of gene fusions in lung cancer that are independent of smoking, we do not understand their pathogenesis.

Our study also focused on diagnostics, realizing that an accurate and precise companion diagnostic would be a key to treating the ALK-positive patients. Our group subsequently helped develop FISH as the gold-standard assay, used as the entry screen for crizotinib clinical trials (4). Thus FISH, while challenging, underwent full clinical validation and predicted the very high response rate seen in ALK FISH-positive patients. IHC, although more attractive, required development of more sensitive monoclonal antibodies and optimized conditions. This work took several years, and IHC was granted FDA approval in 2015 (18). These diagnostic approaches have been evaluated and vetted by national and international studies and professional societies (19). As more fusions and mutations have been uncovered, our group has moved to multiplexed assays to optimize cost and utilize limited tissues available in many patients. NGS has been a major focus, and we have recently developed a targeted assay purpose-built to detect fusions (anchored multiplex PCR), especially when only one partner (e.g., ALK) is known (8). As NGS becomes more widely accepted and less expensive, its throughput and “plex” level will likely make it the most attractive approach for diagnostic fusions.

In conclusion, our study showed that unique clinical features can be very helpful in highlighting and identifying rare tumor subtypes. Rare subtypes, such as ALK fusions, which are present in 3% to 5% of lung cancers, are a challenge to study. We hope that our publication helped elucidate the associated clinicopathologic features, accelerated the studies of ALK inhibitors, and ultimately resulted in clinical benefit for these patients. Although prevention is an ultimate goal of cancer research, unfortunately we still do not understand the underlying pathogenesis of ALK-positive cancers.

References

Disclosure of Potential Conflicts of Interest
A.J. Iafrate reports receiving a commercial research grant from Blueprint Medicines; has ownership interest (including patents) in ArcherDX; and is a consultant/advisory board member for Constellation Pharmaceuticals, Chugai Pharmaceutical, and Debiopharm. No other potential conflicts of interest were disclosed.

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CCR 20th Anniversary Commentary: Molecular Pathology of ALK-Rearranged Lung Tumors

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