Seek and Ye Shall Find: Subclonal Anaplastic Lymphoma Kinase Mutations

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Bellini and colleagues demonstrate the importance of next-generation sequencing to uncover subclonal anaplastic lymphoma kinase (ALK) mutations in neuroblastoma. Although the significance of these subclonal aberrations is not yet understood, deep sequencing could identify patients whose tumors may respond to ALK inhibitors. Clin Cancer Res; 21(21): 4747–9. ©2015 AACR.

See related article by Bellini et al., p. 4913

In this issue of Clinical Cancer Research, Bellini and colleagues (1) demonstrate that targeted deep sequencing of neuroblastoma tumors identifies subclonal mutations in the anaplastic lymphoma kinase (ALK) receptor tyrosine kinase that may have gone undetected using conventional Sanger sequencing. Since the original identification of activating somatic mutations in ALK in neuroblastoma, multiple sequencing studies have validated this kinase as the main targetable molecular aberration in this disease (2–5). In neuroblastoma, the majority of ALK mutations are found in the kinase domain and promote constitutive, ligand-independent activation of the receptor. These alterations are sensitive to small molecule inhibitors of ALK and lead to down-regulation of its activity and that of its downstream targets with consequent growth inhibition of neuroblastoma cells (4). ALK is also activated via translocation events in other malignancies, including non–small cell lung cancer, anaplastic large-cell lymphomas, and inflammatory myofibroblastic tumors, where it is thought to contribute to tumorigenesis and progression (6).

In the study by Bellini and colleagues (1), 276 neuroblastoma samples were analyzed by Sanger sequencing, revealing a mutation rate of 4.3% (12 of 276). To confirm the suspicion that conventional Sanger sequencing may not detect subclonal ALK mutations because of the limits of detection and background noise inherent in this technology, the authors resequenced these samples using the more sensitive two-step PCR procedure and HiSeq technology (Illumina). Targeted resequencing of exons 23 and 25, which contain the two most frequently observed mutational hotspots, F1174 and R1275, respectively, identified subclonal ALK mutations (defined as <20% of the cell population, with up to 50% contamination from normal tissue) in an additional 15 tumors, yielding an overall mutation frequency of approximately 10% (27 of 276). ALK mutations at F1174 were observed in 15 samples, 13 with a mutation leading to the amino acid change F1174L, while 2 samples showed F1174C and F1174V with the mutated allele fractions ranging widely, from 0.5% to 40%. The R1275 locus was mutated in 12 cases, 11 with the R1275Q and one with the R1275L mutation; the proportion of cells with the mutated allele ranged from 0.8% to 73%. This wide range of mutated allele fractions was present even when corrected for tumor cell content and chromosome 2p copy-number status.

There was no association between clonal versus subclonal mutations and important clinical prognostic parameters, such as patient age, tumor stage, or outcome (1). Although on univariate analysis there was a worse overall survival in patients whose tumors expressed mutated or amplified ALK, especially those with the F1174 mutation, this was not borne out when other factors were taken into consideration. In multivariate analysis, advanced-stage disease and MYCN amplification were the only independent prognostic variables, underscoring the secondary role of mutated ALK in determining treatment outcome in this patient cohort.

The authors report a close correlation between mutated ALK and amplified MYCN with enrichment of F1174L in tumors showing MYCN amplification (1), attesting to the demonstrated cooperative effect of both alterations in neuroblastoma (7, 8). Although ALK mutations were either clonal or subclonal, MYCN status was homogeneous throughout the tumor cell population. These observations support the general conclusion that MYCN deregulation is the initial event in neuroblastoma tumorigenesis, with ALK mutations occurring later in tumor development. An interesting observation was the percentage of clonal and subclonal ALK aberrations in the MYCN-amplified versus MYCN-non-amplified tumors. The majority of MYCN-amplified tumors (13 of 15) contained only subclonal populations of ALK-mutated cells, while in MYCN-nonamplified tumors the majority of ALK mutations (10 of 12) were clonal and only 2 of 12 contained subclonal ALK (1). This indicates that in most MYCN-nonamplified tumors with an ALK mutation, the kinase aberration appears to be dominant.

The significance of these subclones is far from clear. They could simply reflect the characteristic genetic heterogeneity of neuroblastoma or they could signal the presence of cell populations with the potential to expand and cause relapse (Fig. 1; ref. 9). The first scenario seems less likely, as almost all of the ALK mutations reported in neuroblastoma are activating (6). However, to date,
there is no experimental evidence that tumors containing malignant subclones have different growth properties or respond differently to ALK inhibitors. The numbers of patients used to determine clinical correlations in the Bellini study was relatively small, and repeat tumor biopsies were not available at relapse, eliminating opportunities to track the fate of subclonal ALK mutations that were uncovered in the diagnostic samples. Two earlier studies reported a higher frequency of ALK mutations at relapse, some of which were present at diagnosis but were below the limits of detection using standard sequencing methods (10, 11). For example, Schleiermacher and colleagues (10) compared 54 paired tumors at diagnosis and relapse and identified 14 ALK mutations, 5 of which were not detected at diagnosis. Deep sequencing revealed subclonal ALK mutations in 2 of 4 diagnostic samples. Moreover, in a recently published study by Eleveld and colleagues (11), in which whole-genome sequencing of 23 paired diagnostic and relapse samples was performed, 10 cases with ALK mutations were identified, of which 3 were not detected in the primary tumor. Ultra-deep sequencing and PCR-based methods identified two of the three being present at low frequency in the primary tumor (11). These examples, as well as the reported establishment of a fully ALK-mutated cell line from a primary tumor containing a subclonal ALK-mutated population (10), are suggestive of clonal evolution, but need to be validated in larger cohorts and through experimental methods. Therefore, whether the higher frequency of ALK mutations seen at relapse reflects those already present at diagnosis or whether they emerged during the development of relapse is a key question that needs to be addressed. It is possible that subclonal ALK mutations at diagnosis may not be effectively targeted by standard chemotherapy and therefore continue to evolve, eventually contributing to relapse.

The presence of subclonal ALK mutations at diagnosis becomes especially important in cases with MYCN amplification, where the majority of ALK-mutated cells were subclonal (1). If these patients are to benefit from the administration of an ALK inhibitor at diagnosis, to prevent ALK-mutated clones from becoming dominant at relapse, it is critical that subclonal ALK mutations are not missed during the initial analysis of the tumor. Sanger sequencing has been used widely in clinical laboratories for the analysis of mutations, but its sensitivity is such that only 20% to 30% of mutated alleles in a wild-type background are detected (12). As more sophisticated technologies become available, such as droplet digital PCR and next-generation sequencing techniques with the potential of detecting mutated allele fractions as low as 0.2%, as previously reported by the authors (10), it should be possible to replace Sanger and other conventional methods for identifying subclonal ALK mutations. Most importantly, this enhanced capability will make it possible to accurately track subclonal ALK mutations from diagnosis to relapse through repeated tumor sampling to establish their clinical relevance. For instance, if the

Figure 1. Possible origins of ALK mutations detected at relapse. A, ALK-mutated cells can be clonal and form the dominant transformed population. These mutations can be identified through conventional sequencing methods. At relapse, these clonal populations retain their dominance, although the possibility of their replacement by clones bearing new mutations cannot be excluded. B, ALK-mutated cells are subclonal at diagnosis and could be either eradicated or evolve and expand at relapse to form the dominant transformed population. These mutations at diagnosis can be missed through conventional sequencing and will need to be searched for through advanced sequencing techniques. C, tumors without ALK mutations at diagnosis but in which ALK mutations arise de novo after treatment.

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findings indicate the persistence and expansion of ALK-mutated subclones in a substantial fraction of patients, there would be justification for adding effective new ALK inhibitors early in the treatment course or at relapse. Whatever the outcome, the study by Bellini and colleagues (1) has provided the impetus to study more closely the pathogenic role of neuroblastoma subclones bearing ALK mutations and hence their potential value as therapeutic targets.

References

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