Editorial

The reality of complexity: concomitant genomic alterations in patients with *EGFR* mutations

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The advent of next-generation sequencing in the past years has allowed us to unearth a mass amount of information regarding diverse pathologies. Among these, the sequencing of cancer genomes has identified a number of oncogenic alterations which could strongly impact our understanding of tumor biology as well as our current treatment modalities. However, even with the growing availability of this technology most sequencing studies are often performed in patients with early-stage tumors, in spite that it is the patients with advanced tumors who are generally treated with targeted therapies. Lung cancer remains the main cause of cancer-related deaths worldwide (1), and patients diagnosed with advanced lung cancer may have multiple and sometimes rare genetic alterations (2); currently there is little information regarding the effects of co-occurring genetic alterations. Previously, others (3-5) have explored the role of the non-disruptive p53 mutations in *EGFR* mutated lung adenocarcinoma patients finding a prevalence close to 30–50% with negative impact on the prognosis, especially in those with exon 19 deletions. Similarly, Hu *et al.* evaluated the presence of mutations in *KRAS*, *NRAS*, *PIK3CA*, *BRAF*, and *HER2* and *ALK*, *ROS1*, and *RET* fusion genes in 320 patients who harbored EGFR activating mutations and received EGFR-tyrosine kinase inhibitor (TKIs) treatment (6). Twenty-one (6.6%) of the *EGFR* mutant samples had additional gene alterations, being the most common those found in *PIK3CA*, followed by *EML4-ALK* rearrangement, *HER2* mutations, *RET* rearrangement, *ROS1* rearrangement and *KRAS* mutations. Those with a single *EGFR* mutation had a significantly longer progression-free survival (PFS) compared to those with concurrent gene alterations, however, this condition did not have a significant impact in the OS.

Overcoming tumor heterogeneity is a major challenge for the personalized treatment of cancer. Although intratumoural heterogeneity has been well described in a variety of cancer types (7,8), including non-small cell lung cancer (NSCLC) (9), the degree to which tumor heterogeneity currently influences treatment decisions in the clinic remains limited. Despite some evidence that multiple resistant subclones can arise following treatment of NSCLC patients with EGFR-targeted therapies (10-12), the fraction of patients who develop multiple resistance mechanisms has not been systematically evaluated. This is largely because almost all the studies performed to date have relied on tissue biopsies that are limited by the presence of geographic heterogeneity. Analysis of ctDNA has advantages over traditional biopsies in that the procedure is minimally invasive, can detect contributions from multiple tumor deposits, and can easily be repeated

over time, allowing a more comprehensive analysis of tumor heterogeneity (13). In addition, we are now aware of the performance of liquid biopsies to characterize the presence of mutations in *EGFR*, knowing that ctDNA provides high specificity, but nonetheless acknowledging that patients with negative results by this method should decidedly undergo further biopsies to ascertain to comprehensive mutational analysis (14).

To determine if co-occurring alterations cooperate with the primary tumor driver to promote tumor progression and response to therapy, Blakely *et al.* (15) sequenced cell-free DNA (cfDNA) from 1,122 patients with *EGFR*-mutant advanced NSCLC and 1,008 patients with *EGFR* mutation-negative advanced NSCLC. The *EGFR*-mutant patient cohort had widespread occurrence of additional genetic alterations (92.9 percent had at least one other cancer-related variant) including mutations in *PIK3CA*, *BRAF*, *MET*, *MYC*, *CDK6*, and *CTNNB1*. Further, the *EGFR*-mutant samples had an enrichment of certain genetic events, including co-alterations in *CTNNB1*, *CDK6*, or *AR*, compared with the *EGFR* mutant-negative samples. Patients with the *EGFR* p.Thr790Met mutation, which confers resistance to some EGFR TKIs, had enrichment of a number of co-occurring alterations including *CDK6*, *CTNNB1*, *AR*, *MYC*, *KRAS*, and *PDGFRA*. Longitudinal data were available for 97 patients with *EGFR*-mutant tumors, and analysis of these samples revealed acquisition of the *EGFR* p.Thr790Met mutation upon resistance to first-generation EGFR TKIs, and co-alterations in *CCNE1*, *NF1*, and *PIK3CA* after progression to second-line therapy. EGFR TKI responders had fewer cfDNA mutations than non-responders. Co-occurring mutations associated with primary resistance to the third-generation EGFR-TKI osimertinib were also observed. Collectively, these findings reveal that co-occurring genetic alterations are linked to therapeutic response and resistance in *EGFR*-mutant lung cancer and demonstrate that more detailed genomic information is required to understand tumor heterogeneity beyond a single driving oncogene. Another such example of the complexity regarding tumor biology and treatment outcomes was seen in the recent study by Campos-Parra *et al.*, which showed that KRAS mutation status is a good biomarker for response to therapy with EGFR-TKIs, highlighting the complexity of the pathways which might be necessary to overcome for a successful therapy (16).

In perspective, for us as clinicians and researchers it is remarkable to discover that half the patients had mutations in the *TP53* gene while alterations in the *CTNNB1*, *CDK6*, and *AR* genes were also enriched among those patients. At the pathway level, alterations affecting WNT/CTNNB1 and hormone signaling protein pathways were enriched among patients with *EGFR*-mutant tumors. Additionally, Blakely *et al.* noted that alterations in genes involved in cell division, epigenetic modifications, DNA repair, and cellular signaling pathways were also affected in 10% to 25% of tumors. For 97 of the patients in the study, the researchers also had clinical and treatment response data. This subset included patients who were TKI treatment-naïve as well as those who showed progression on first-line inhibitors or on second-line compounds. The researchers noted that, with each line of therapy, the number of somatic mutations in the patients' samples increased. In the same way, patients who progressed after receiving second-line TKIs tended to have an increased number of co-mutations in *CCNE1*, *NF1*, and *PIK3CA*, and increased alterations in receptor tyrosine kinase, MAPK, and cell-cycle pathways. Additionally, the researchers noted that alterations within the cell-cycle and MAPK pathways were markers of shorter PFS during EGFR-TKI treatment, and patients with CDK4 or CDK6 alterations had lower overall survival than those without these alterations. Blakely and his colleagues also analyzed tumor exome and cfDNA samples that had been collected from an NSCLC patient over the course of 6 years. Within this patient, they uncovered multiple co-alterations that cropped up early on. For instance, early-stage disease samples harbored alterations in *CTNNB1*, *SMAD4*, and *CDKN2A*, while mutations arose in *PRKCA* and *PIK3CA*, among others, after metastasis. Progression on TKI then occurred following acquisition of the *EGFR* p.Thr790Met mutation.

This data confirms the value of serial genomic profiling to detect resistance mutations as early as 16 weeks before radiographic progression (17). There is a potential for important monetary savings when ineffective use of expensive new anticancer drugs is avoided or halted. Challenges for routine implementation of liquid biopsy tests include the necessity of specialized personnel, instrumentation, and software, as well as further development of quality management (external quality control). Validation of blood-based tumor genomic profiling in additional multicenter outcome studies is necessary; however, in the near future cfDNA monitoring will provide clinically important actionable information for precision oncology approaches. For the moment, the question remains the reality of complexity. What is the next step? What is the predictive role of the concurring mutations in

patients with EGFR alterations? Are we facing a new era that includes diverse multidrug combinations? Only time will tell us the complexity of reality.

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Footnote

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

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