Next-Generation Sequencing: Targeting Targeted Therapies

Justine N. McCutcheon and Giuseppe Giaccone

In this issue of Clinical Cancer Research, Drilon and colleagues (1) demonstrate the significant role of next-generation sequencing (NGS) as the primary testing method in molecular diagnostics. Thirty-one previously tested lung adenocarcinoma patients assessed by single non-NGS molecular tests, such as fluorescence in situ hybridization (FISH), multiplex mass spectrometry, and sizing assays, produced "negative" results for known lung adenocarcinoma genomic alterations in the genes EGFR, ERBB2, KRAS, NRAS, BRAF, MAP2K1, PIK3CA, AKT1, ALK, ROS1, and RET. By retesting these patients with a broad, hybrid capture-based NGS assay, Drilon and colleagues (1) revealed actionable genomic alterations present in 65% of the patients that were formerly deemed "driver negative."

NGS technologies are rapidly evolving and are being increasingly used in research settings as well as clinical settings, to replace older and less-sensitive technologies. As opposed to classic Sanger sequencing, NGS technology allows the patient's tumor to be tested in a single run. NGS technologies use clonally amplified or single-molecule templates that are sequenced in a massively parallel fashion, allowing examination of numerous amounts of large protein coding regions, which makes NGS assays suitable for a comprehensive interrogation of cancer drivers (2). Whereas current non-NGS tests mostly examine only one variant type, NGS technology allows the patient's tumor to be tested in a single run for all types of variants, including single-nucleotide variations (SNV), insertions, deletions, exon duplications, gene copy number changes, and known translocations (3).

In recent years, the advancement of NGS technology in the clinical setting has been rapidly progressing and will soon likely replace older technologies. Lung cancer, the leading cause of cancer-related death in the world, comprises a complex mutational spectrum, and the discoveries of many oncogenic drivers in the tumors have led to the development and evolution of targeted therapy, especially in the adenocarcinoma of the lung, the most prevalent type of lung cancer (4, 5). Among these targeted therapies are the tyrosine kinase inhibitors (TKI); the FDA approved TKIs in lung adenocarcinoma treatment to target EGFR and ALK. Sensitizing mutations and rearrangements in the EGFR and ALK genes, respectively, are responsible for the constitutively activated kinase, and render tumors exquisitely sensitive to TKIs. In the case of EGFR and ALK inhibitors, response rates and progression-free survival are dramatically improved compared with standard chemotherapy (6). Although TKIs are initially very effective in the majority of patients whose tumors harbor the genetic alteration, acquired resistance invariably occurs. Often additional mutations are found, which are responsible for this resistance, such as the T790M mutation found in the EGFR gene. Third-generation EGFR inhibitors and second-generation ALK inhibitors are active in the presence of resistant mutations (7–9). TKI-sensitive and TKI-resistant mutations can be identified through an NGS assay within a single run.

We are now in the era of personalized medicine or personalized patient care in oncology: customized healthcare tailored to an individual patient based on the genetic information obtained from the patient's tumor. Drilon and colleagues (1) demonstrate how NGS technology plays a role in personalizing patient care in non–small cell lung cancer treatment by showing evidence that current non-NGS molecular diagnostic tests failed to detect known genomic alterations in patients who, in actuality, possessed these alterations. Furthermore, 39% of these patients' genomic alterations had a targeted agent accessible through a clinical trial, and 6 of these patients received targeted therapy with a beneficial outcome of either partial response or evidence of disease shrinkage.

Other clinical benefits from using an NGS assay over non-NGS tests are the reduced patient sample consumption. Most NGS assays require as little as 10 ng of DNA, whereas non-NGS tests, like FISH and IHC tests, require several histologic sections of the formalin-fixed, paraffin-embedded (FFPE) specimen block for a single run (10). As described by Drilon and colleagues (1), 84% of the patients needed an additional biopsy due to their original biopsy specimen having insufficient tissue for the NGS assay. Additionally, of those patients, 69% already endured multiple biopsies for non-NGS tests alone. As with any surgical procedure, multiple biopsies performed on patients increases the risk of complications and are expensive. Non-univocal testing results can also occur from numerous testing runs from various parts of the same tumor, representing tumor heterogeneity.

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doI: 10.1158/1078-0432.CCR-15-0407

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Even so, having a new and different assay try to take over standard clinical tests for tailoring of targeted therapy is an arduous task for hospitals and cancer centers. Including but not being limited to learning the new assay, validating the tests and training personnel, healthcare centers must examine FDA and clinically approved regulations, insurance policies, and cost-efficiency strategies. In a recent paper, overall patients were more willing to undergo molecular testing if it is an approved therapy and is covered by insurance (11). Healthcare centers need to relay all the valuable outcomes of testing with an NGS assay to their patients to eliminate the possibility of uncertainty. Genomic companies, such as Foundation Medicine, which has been used in this work by Drilon and colleagues, have developed and manufactured genomic analysis diagnostic tests for solid tumors and hematologic malignancies using NGS, with a relatively rapid turnaround time of sequencing data. Moreover, the costs of NGS instruments and reagents have been significantly decreasing in the last years, making it a more attractive option to healthcare centers of middle to large size to implement the technology in their own facilities. Major manufacturers of sequencing instruments, such as Illumina and Ion Torrent (Life Technologies), are constantly improving and increasing the accuracy and quality of sequencing reads, data output, and turnaround time, while making a variety of instruments commercially available at a reasonable price. For example, the MiSeq and the Ion Personal Genome Machine (PGM) are both small benchtop sequencers that yield up to 15 gigabases (Gb) and 2 Gb with an approximate turnaround time of 2.5 days and 8 hours, respectively (Table 1). Although NGS assays are still in the early stages of becoming a standard, clinically approved, regulated test, many cancer and medical centers across the United States are already offering these tests in house to patients, and the sequencing industry is slowly integrating cancer panels into their FDA-approved platforms (12).

Having an assay as such provides clinicians the ability to "personalize" each patient’s treatment by assessing the patient’s genomic mutations and administering a drug that will deliver an improved outcome. Drilon and colleagues have revealed the specificity and comprehensiveness of using a broad, hybrid capture-based NGS assay and implemented it into their own facility at the Memorial Sloan Kettering Cancer Center. Although it will still take some time for NGS to become a broadly accepted standard clinical test, we have no doubt that this technology will replace older technologies in the near future.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: J.N. McCutcheon, G. Giaccone
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Received March 11, 2015; accepted March 24, 2015; published OnlineFirst April 6, 2015.

Table 1. Throughput and run times of the two most widely used NGS instrument brands

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<thead>
<tr>
<th>Instrument</th>
<th>Maximum data yield</th>
<th>Maximum run time</th>
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<tbody>
<tr>
<td>HiSeq 4000</td>
<td>1,500 Gb</td>
<td>3.5 days</td>
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<tr>
<td>HiSeq 3000</td>
<td>750 Gb</td>
<td>1.5 days</td>
</tr>
<tr>
<td>HiSeq 2500</td>
<td>1,000 Gb</td>
<td>6 days</td>
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<tr>
<td>NextSeq 500</td>
<td>120 Gb</td>
<td>29 hours</td>
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<tr>
<td>MiSeq</td>
<td>15 Gb</td>
<td>55 hours</td>
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<tr>
<td>Ion Torrent (Life Technologies) Ion Proton</td>
<td>10 Gb</td>
<td>4 hours</td>
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References
