Next-Generation Sequencing: Targeting Targeted Therapies

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**Running Title:** Next-Generation Sequencing as a Clinical Assay

**Disclosure of Potential Conflicts of Interest**

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Summary

Next-generation sequencing (NGS) has given new perspective in oncology. With the ongoing development of targeted therapies, NGS is evolving molecular diagnostics by providing comprehensive interrogation of clinically-actionable genomic aberrations in tumors. Having this assay as the primary testing method produces clinically beneficial results.
In this issue of *Clinical Cancer Research*, Drilon and colleagues 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 revealed actionable genomic alterations present in 65% of the patients that were formerly deemed “driver negative” (1).

Next-generation sequencing (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 classical Sanger Sequencing, NGS technology uses 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 (SNVs), 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 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 adenocarcinoma of the lung, the most prevalent type of lung cancer (4, 5). Among these targeted therapies are the tyrosine kinase inhibitors (TKIs); the FDA approved TKIs in lung adenocarcinoma treatment target the epidermal growth factor receptor (EGFR) and the anaplastic lymphoma kinase (ALK). Sensitizing mutations and rearrangements in the $EGFR$ and $ALK$ gene, 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 to 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 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 demonstrate how NGS technology plays a role in personalizing patient care in NSCLC treatment by showing evidence that current non-NGS molecular diagnostic tests failed to detect known genomic alterations in patients that, in actuality, possessed these mutations. 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, while non-NGS
tests, like FISH and immunohistochemistry (IHC) tests, require several histological sections of
the formalin-fixed, paraffin-embedded (FFPE) specimen block for a single run (10). As described
in Drilon and colleagues’ paper, 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.

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 like 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 hematological malignancies using NGS,
with a relatively rapid turnaround time 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, whilst making a variety of instruments
commercially available to laboratories at a reasonable price. For example, the MiSeq and the
Ion Personal Genome Machine (PGM) are both small bench top 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 Memorial Sloan Kettering Cancer Center. Although it will take still 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.

References


Table 1. Throughput and run times of the two most widely used next-generation sequencing instrument brands

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<th>Illumina</th>
<th>Ion Torrent (Life Technologies)</th>
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<tbody>
<tr>
<td></td>
<td>HiSeq 4000</td>
<td>HiSeq 3000</td>
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<tr>
<td>Maximum Data Yield</td>
<td>1500 Gb</td>
<td>750 Gb</td>
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<tr>
<td>Maximum Run Time</td>
<td>3.5 days</td>
<td>3.5 days</td>
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<tr>
<td></td>
<td>HiSeq 2500</td>
<td>NextSeq 500</td>
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<td></td>
<td>1000 Gb</td>
<td>120 Gb</td>
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<td>6 days</td>
<td>29 hours</td>
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<td>MiSeq</td>
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<td>55 hours</td>
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<td>Ion Proton</td>
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<td></td>
<td></td>
<td>10 Gb</td>
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<td></td>
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<td>4 hours</td>
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<td></td>
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<td>Ion PGM</td>
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<td></td>
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<td>2 Gb</td>
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<td>7.3 hours</td>
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Abbreviations: Gb, gigabase; PGM, personal genome machine.
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