



Next-Generation DNA Sequencing: Implications for Oncology Care

Suzanne M. Mahon, RN, DNSc, AOCN®, APNG

Genetic testing for germline hereditary predisposition syndromes usually involves traditional Sanger DNA sequencing (see Figure 1). New advances in genomic technologies have led to reduced cost and turnaround time with the simultaneous testing of multiple genes. This, in turn, has led to the introduction of next-generation sequencing (NGS) panels that analyze less common high- and intermediate-penetrance cancer susceptibility genes. The ultimate goal of NGS is to reduce the cost of whole genome sequencing to about \$1,000 and provide robust and comprehensive information about hereditary risk for developing a myriad of diseases (Rizzo & Buck, 2012).

NGS is quite different from direct-to-consumer (DTC) genetic testing. DTC genetic testing includes tests that are marketed directly to the individual and can be performed without the inclusion of a physician, genetics professional, pre- and post-test counseling, or an insurance company (Hudson, Javitt, Burke, Byers, & ASGH Social Issues Committee, 2007). Many of these tests reportedly can assess for future risk of multiple diseases. DTC typically involves analyzing hundreds to thousands of single nucleotide polymorphism (SNP) chips to simultaneously examine thousands of small changes found across the genome. Some of these SNPs are known to be associated with a disease, although the exact clinical implications, penetrance, and disease risk conferred by many of these SNPs are unclear.

Laboratories that perform clinical genetic testing must be certified by the Clinical Laboratory Improvement Amendments (CLIA) of 1988. However, many of the laboratories offering DTC testing do not disclose their CLIA certification. NGS typically is carried out in

CLIA-approved laboratories, involves genes that have known associations with disease susceptibility, and uses different laboratory techniques.

Laboratory Techniques

Sanger sequencing has evolved from the original sequencing technique that used the manual chain-termination sequencing method to automated sequencing instruments that detect fluorescently labeled nucleotide sequences, and it was the method used to sequence the first human genome (Ross & Cronin, 2011). It often is referred to as first-generation sequencing (Rizzo & Buck, 2012). Automation has been accompanied by decreased costs and turnaround time for results (see Figure 2). Despite these advances, the main limitation of Sanger sequencing is that only a limited amount of data can be read with each sequence reaction (Rizzo & Buck, 2012).

More recently, efforts have been underway to increase efficiency by sequencing massive numbers of different DNA sequences in a single reaction, which is called a parallel reaction. This is referred to as NGS or massive parallel DNA sequencing (Desmedt, Voet, Sotiriou, & Campbell, 2012; Rizzo & Buck, 2012).

In the future, whole exome and whole genome sequencing are anticipated to become the gold standard in genetic testing. Although 100% of the human genome has been sequenced, only about 10% has been characterized (i.e., all possible genomic alterations and mutations identified), so doing whole genome sequencing is currently of limited clinical utility (Rizzo & Buck, 2012). Therefore, only sequencing the exome, which comprises the 2% of the genome denoted by protein-coding regions known as exons

and is associated with coding regions of about 3,000 known diseases, is more efficient (Rizzo & Buck, 2012).

Clinicians already are using targeted exome sequencing to make clinical diagnoses, including panels to identify cancer susceptibility genes (Biesecker, Burke, Kohane, Plon, & Zimmern, 2012; Ku, Cooper, Iacopetta, & Roukos, 2013). For example, Ambry Genetics Laboratories offers several next-generation cancer panels. CancerNext™ is an NGS panel that simultaneously analyzes 22 genes that contribute to increased risk for breast, colon, ovarian, uterine, and other cancers. Ambry Genetics Laboratories (n.d.) also offers similar tests specifically for breast, ovarian, and colon cancers.

The National Comprehensive Cancer Network (NCCN), 2013 has just updated its recommendations to include NGS for hereditary breast, ovarian, and other cancers. However, because of the complexity and variety of results interpretation, NCCN states these panels should only be ordered in consultation with a genetics professional.

Implications for Clinical Care

The implications of NGS for clinical care should not be underestimated. The American College of Medical Genetics and Genomics (2012) issued a policy statement on the use of NGS that emphasized the importance of correctly identifying families likely to benefit from testing, comprehensive pretest counseling, post-test considerations, and the role of genetics professionals. Clearly, genetics professionals as well as other healthcare providers will require education about NGS, how to manage results, and the interpretation of large amounts of genetic data (Rizzo & Buck, 2012).

- ▶ **Characterization:** identification of the full set of genomic alterations (e.g., deletions, insertions, translocations) within a gene that cause a disease-associated mutation
- ▶ **DNA sequencing:** process of determining the precise order of the nucleotides (adenine, guanine, cytosine, and thymine) within a DNA molecule
- ▶ **Exome:** part of the genome formed by exons, which are the sequences that, when transcribed, remain within the mature RNA after the introns are removed by RNA splicing
- ▶ **Genome:** complete set of genetic material of an organism, including the total genetic content in one set of chromosomes that includes all the inheritable traits in an organism
- ▶ **Germline:** inherited genetic material that is passed down to the offspring through the egg or sperm before it is modified by somatic recombination or maturation
- ▶ **Massively parallel sequencing:** not a test in itself or a specific sequencing technology; rather, the term emphasizes a distinction from initial approaches that involved the sequencing of one DNA strand at a time
- ▶ **Next-generation sequencing:** sequencing in which many strands of DNA are sequenced at once, generating far more data per instrument run than the Sanger method
- ▶ **Sanger sequencing:** original sequencing technology that helped scientists determine the human genetic code. Now automated, it still is used to sequence short pieces of DNA. It relies on a technique known as capillary electrophoresis, which separates fragments of DNA by size and then sequences them by detecting the final fluorescent base on each fragment. Named after the Sanger Institute in England.
- ▶ **Single nucleotide polymorphisms (SNPs):** each SNP (pronounced “snip”) represents a difference in a single nucleotide. SNPs occur normally throughout a person’s DNA, usually about once in every 300 nucleotides (roughly 10 million SNPs in the human genome). These variations often are found in DNA between genes functioning as biologic markers, helping scientists locate genes associated with disease. When SNPs occur within a gene or in a regulatory region near a gene, they may play a more direct role in disease by affecting the gene’s function.
- ▶ **Whole exome sequencing:** involves determination of the DNA sequence of most of the protein-encoding exons and may include some DNA regions that encode RNA molecules not involved in protein synthesis. In some cases, exome testing may be targeted to particular genes, such as a panel that targets genes associated with hereditary cancer syndromes.
- ▶ **Whole genome sequencing:** involves determination of the sequence of most of the DNA content comprising the entire genome of an individual

Figure 1. Definitions of Common Terms

Note. Based on information from Ku, Cooper, Iacopetta, et al., 2013; Meldrum et al., 2012; O’Daniel & Lee, 2012; Ross & Cronin, 2011.

As the ability to analyze many hereditary cancer syndromes in one comprehensive test becomes a reality, testing costs and turnaround time will continue to decrease. In terms of complete exome or genome sequencing, an individual might learn of disease risk that they did not anticipate, such as dementia or another disease for which no prevention or treatment exists. Such an unexpected incidental finding also may have implications for other family members. In targeted-cancer NGS, the platform focuses exclusively on known causal genes so the issue of the disclosure of incidental findings is not as likely to occur (Ku, Cooper, Ziogas, et al., 2013).

The risk of a variant of unknown significance may be higher in NGS, as less is known about some of the genes (Ku, Cooper, Ziogas, et al., 2013). For example, CancerNext includes testing for the following genes: *APC*, *ATM*, *BARD1*, *BRIP1*, *BMPR1A*, *CDH1*, *CHEK2*, *EPCAM*, *MLH1*, *MRE11A*, *MSH2*, *MSH6*, *MUTYH*, *NBN*, *PALB2*, *PMS2*, *PTEN*, *RAD50*, *RAD51C*, *SMAD4*, *STK11*, and *TP53* (Ambry Genetics Laboratories, n.d.). The clinical implications of a mutation in some of those genes are poorly understood. In addition, recognizing intermediate-penetrance (two- to five-fold increased risk) genes, their contribution to cancer, and the subsequent management of mutation carriers continues to pose a challenge in clinical management because of a lack of published guidelines.

Many hereditary cancer syndromes have confusing clinical presentations and it can be challenging to select the correct test. For example, Lynch syndrome and familial adenomatous polyposis (FAP) have some overlapping clinical features. As both attenuated FAP and Lynch syndrome are characterized by a similar median age of onset and comparable numbers of colonic polyps, it may not be clear which genetic test to order first. Simultaneous testing for germline mutations associated with colon cancer using a NGS targeted colon platform may be more efficient and cost effective (Ku, Cooper, Iacopetta, et al., 2013). NGS might also be useful in people who have been adopted or have limited

information about their family history (Meldrum, Doyle, & Tothill, 2011; O’Daniel & Lee, 2012).

Another clinical application of targeted-cancer NGS platforms occurs with genetic testing for hereditary breast cancer. Many mistakenly believe that if a woman tests negative for a mutation in *BRCA1* or *BRCA2* that no hereditary risk of breast cancer exists, but *BRCA1/2* probably only account for about 25% of hereditary breast cancer (Desmedt et al., 2012). In the absence of a known mutation, such a negative result only implies that the woman does not have a mutation in *BRCA1/2*. At least 19 other odd hereditary syndromes could be associated with breast cancer (Walsh et al., 2010). NGS has the potential to detect these other lower penetrance genes. One commercially available targeted panel that tests for 14 of these genes is called Breast Next™ (Ambry Genetics Laboratories, n.d.). It typically is ordered after a negative *BRCA1/2* test and may account for as much as 20% of hereditary breast cancer (Ambry Genetics Laboratories, n.d.).

Ethical challenges also are associated with NGS. Although the targeted sequencing approach is less complicated in terms of data analysis and interpretation, ethical concerns still remain (Ku, Cooper, Ziogas, et al., 2013), including issues surrounding how NGS findings are disclosed, how the information is used in clinical care, and establishment of insurance reimbursement protocols for NGS testing (Rizzo & Buck, 2012).

The evolution of NGS technologies is facilitating and changing research in cancer genetics and genomic science (Ku, Cooper, Ziogas, et al., 2013). NGS undoubtedly will lead to a better understanding of the natural history of many cancer predisposition syndromes, identification of other susceptibility genes, and the interaction between genes, as well as ultimately guiding therapeutic decisions.

Technologic developments will continue in the field of genomics. Oncology nurses need to understand not only the technology and biology underlying genetic testing, but also the significance of the clinical applications of new technologies,

Traditional Sanger Sequencing

- Less technical and intellectual expertise is needed for data analysis.
- Turnaround time is shorter.
- Testing is for a specific gene so counseling can be more straightforward and directed.

Next-Generation Sequencing

- Costs less than multiple individual tests
- Can sequence multiple genes at one time from one sample
- Can detect deletions, translocations, insertions, and copy number alterations
- Potential for greater sensitivity
- Potential for greater specificity
- Can be useful in clinically confusing presentations when an obvious syndrome is unclear, enabling the testing of multiple genes simultaneously rather than sequentially

Figure 2. Advantages of Traditional Sanger Sequencing and Next-Generation Sequencing

Note. Based on information from Ku, Cooper, Iacopetta, et al., 2013; O'Daniel & Lee, 2012; Ross & Cronin, 2011.

including NGS. Oncology nurses will continue to be challenged to correctly identify patients who might benefit from genetic counseling and possibly genetic testing and refer them to a genetics professional who is familiar with new technologies such as NGS.

Suzanne M. Mahon, RN, DNSc, AOCN®, APNG, is a professor in the Department of Internal Medicine and the School of Nursing at Saint Louis University in Missouri. No financial relationships to disclose. Mahon can be reached at mahonsm@slu.edu, with copy to the editor at ONF Editor@ons.org.

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References

- Ambry Genetic Laboratories. (n.d.). Hereditary cancer panels. Retrieved from <http://www.ambrygen.com/hereditary-cancer-panels>
- American College of Medical Genetics and Genomics. (2012). *Policy statement: Points to consider in the clinical application of genomic sequencing*. Retrieved from <http://bit.ly/18h4mWq>
- Biesecker, L.G., Burke, W., Kohane, I., Plon, S.E., & Zimmern, R. (2012). Next-generation sequencing in the clinic: Are we ready? *Nature Reviews. Genetics*, 13, 818–824. doi:10.1038/nrg3357
- Desmedt, C., Voet, T., Sotiriou, C., & Campbell, P.J. (2012). Next-generation sequencing in breast cancer: First take home messages. *Current Opinion in Oncology*, 24, 597–604.
- Hudson, K., Javitt, G., Burke, W., Byers, P., & ASHG Social Issues Committee. (2007). ASHG Statement* on direct-to-consumer genetic testing in the United States. *American Journal of Human Genetics*, 81, 635–637. doi:10.1086/521634
- Ku, C.S., Cooper, D.N., Iacopetta, B., & Roukos, D.H. (2013). Integrating next-generation sequencing into the diagnostic testing of inherited cancer predisposition. *Clinical Genetics*, 83, 2–6.
- Ku, C.S., Cooper, D.N., Ziogas, D.E., Halkia, E., Tzaphlidou, M., & Roukos, D.H. (2013). Research and clinical applications of cancer genome sequencing. *Current Opinion in Obstetrics and Gynecology*, 25, 3–10.
- Meldrum, C., Doyle, M.A., & Tothill, R.W. (2011). Next-generation sequencing for cancer diagnostics: A practical perspective. *Clinical Biochemist Reviews*, 32, 177–195.
- National Comprehensive Cancer Network. (2013). *Genetic/familial high-risk assessment: Breast and ovarian* [v.3.2013]. Retrieved from http://www.nccn.org/professionals/physician_gls/pdf/genetics_screening.pdf
- O'Daniel, J.M., & Lee, K. (2012). Whole-genome and whole-exome sequencing in hereditary cancer: Impact on genetic testing and counseling. *Cancer Journal*, 18, 287–292.
- Rizzo, J.M., & Buck, M.J. (2012). Key principles and clinical applications of “next-generation” DNA sequencing. *Cancer Prevention Research*, 5, 887–900.
- Ross, J.S., & Cronin, M. (2011). Whole cancer genome sequencing by next-generation methods. *American Journal of Clinical Pathology*, 136, 527–539.
- Walsh, T., Lee, M.K., Casadei, S., Thornton, A.M., Stray, S.M., Pennil, C., . . . King, M.C. (2010). Detection of inherited mutations for breast and ovarian cancer using genomic capture and massively parallel sequencing. *Proceedings of the National Academy of Sciences*, 107, 12629–12633.

Genetics & Genomics

This feature aims to educate oncology nurses about the emerging role of genetics and genomics in cancer care. Possible submissions include, but are not limited to, application of genetics and genomics in clinical practice, screening and surveillance, case studies to present new ideas or challenge current notions, and ethical issues. Manuscripts should

clearly link the content to the impact on cancer care. Manuscripts should be 1,000–1,500 words, exclusive of tables and figures, and accompanied by a cover letter requesting consideration for this feature. For more information, contact Associate Editor Lisa B. Aiello-Laws, RN, MSN, AOCNS®, APN-C, at lba34@drexel.edu.