An Introduction to Next-Generation Sequencing for Oncologists

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I. Genomics in Clinical Oncology

The introduction of genomic technologies transformed the way biologists think about human health and disease. After the Human Genome Project constructed the first human reference genome sequence in 2003,[1] years of cancer research have discovered genetic mutations associated with various diseases, including cancer. To date, at least 138 known cancer-related genes have been discovered—74 tumor suppressors and 64 oncogenes—that drive tumor growth through 12 known cellular signaling pathways.[2]

> "As costs continue to come down, we are entering a period where we are going to be able to get the complete catalog of disease genes. This will allow us to look at thousands of people and see the differences among them, to discover critical genes that cause cancer, autism, heart disease, or schizophrenia."
>
> —Eric S. Lander, PhD, founding director of the Broad Institute of MIT and Harvard and a principal leader of the Human Genome Project[3]

Increasingly, advances in genomic technologies are used to understand the genetic changes driving cancer progression. Next-generation sequencing (NGS) is arguably the most significant of these advances. Practicing oncologists are beginning to consider certain sequencing-based diagnostics to guide clinical actions, such as the choice of drugs or drug combinations. In fact, several of the leading cancer centers, such as the Dana Farber Cancer Institute and the Mayo Clinic, have announced that they are now prepared to sequence "all patients for millions of tumor mutations."[4,5]

> "It is easy to imagine that soon every patient will have both their constitutional and cancer genomes sequenced...thus enabling an accurate molecular subtyping of disease and the rational use of molecularly guided therapies."
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> —Cliff Meldrum, Maria A. Doyle, and Richard W. Tothill[6]

II. What is Next-Generation Sequencing?

NGS is a high-throughput process that determines the DNA sequence of an individual. This method is highly scalable—it can be applied to a subset of key genes or the entire genetic code. In principle, the concept behind NGS technology is similar to Sanger sequencing. DNA polymerase catalyzes the incorporation of fluorescently labeled nucleotides into a DNA template strand during sequential cycles of DNA synthesis. During each cycle, the nucleotides are identified by fluorophore labels. The critical difference is that instead of sequencing a single DNA fragment, NGS extends this process across millions of fragments in a massively parallel fashion.

NGS offers several advantages over existing methods such as PCR amplicon testing and Sanger sequencing. Because NGS can assess multiple genes in a single assay, it eliminates the need to order multiple tests to identify the causative mutation. This multigene approach decreases the time to answer, providing a more economical solution and reducing the risk of exhausting precious clinical samples. In addition, NGS can provide high sensitivity, enabling the detection of mutations present at as little as 5% of the DNA isolated from a tumor sample.

III. Advantages of Next-Generation Sequencing

Compared to traditional methods, NGS offers advantages in accuracy, sensitivity, and speed that can make a significant impact on the field of oncology in the future.

Tumor Profiling With Targeted Sequencing

The advent of molecular profiling overcame the limitations of traditional solid tumor classification methods, which relied on the morphology of tumor cells and the surrounding tissue. Today, molecular profiling is a standard technique for classifying tumors, with established guidelines from the College of American Pathologists[7] and the National Comprehensive Cancer Network.[8] Molecular profiling is critical for identifying and characterizing the unique somatic mutations that accrue in cancer cells.

In turn, genomic technology has evolved to meet molecular profiling needs. Tumor profiling using NGS focuses on a preselected subset of genes (also known as a gene panel). These panels contain genes that have known involvement in cancer, enabling the assessment of all potentially causative genes at the same time. Tumor profiling using NGS follows a simple workflow that can be easily scaled to hundreds of samples, enabling clinical labs to process more samples and deliver answers sooner.

Targeted gene sequencing offers several advantages:

- Analyzes multiple genes in a single assay
- Optimizes use of limited tissue samples by reducing need for sequential testing
- Enables the accurate identification of rare variants in heterogeneous tumor samples

Liquid Biopsies

Cell-free, circulating tumor DNA (ctDNA) can act as a noninvasive cancer biomarker, offering a potential alternative to invasive tissue biopsies. Today, researchers are investigating the use of ctDNA as a biomarker for detecting the presence of tumors in "liquid biopsies" obtained through a simple blood draw.[9–11] In the future, ctDNA could potentially serve as a noninvasive approach for real-time cancer detection, monitoring of therapeutic response, assessing remission or progression, and screening for disease. NGS offers the sensitivity and specificity needed to detect low levels of ctDNA in the bloodstream. Further refinement of this technology and the development of assays for ctDNA detection hold considerable potential to revolutionize the way cancer is identified and treated, leading to earlier diagnosis, improved survival rates, and better quality of life for cancer patients.

> "The holy grail for the field is using these types of noninvasive approaches for early detection."
>
> —Victor Velculescu, MD, PhD, Professor of Oncology and Co-Director of Cancer Biology, Johns Hopkins University School of Medicine[12]

IV. Summary

Over the last decade, advances in genomics have led to an improved understanding of cancer biology, which, in turn, has led to new approaches to managing the disease. The adoption of sequencing-based tests continues to grow, reducing time and costs for molecular testing procedures and offering the potential for more specific, individualized patient assessment.

Illumina is committed to providing the highest-quality data in the industry, exemplified by implementation of the largest instrument install base of any NGS technology company[13] and its relationships with leaders in the oncology field. Together, we will bring the promise of NGS toward widespread clinical adoption and toward improvements in patient diagnosis, treatment, and outcomes.

V. References

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