

CLINICAL APPLICATIONS OF NEXT-GENERATION SEQUENCING

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ABSTRACT

The significant drop in sequencing costs boosted by chemistries optimization and sample multiplexation has resulted in an immense growth within the field of next-generation sequencing (NGS) in the last decade. This has allowed a diversification of techniques, promoting a rapid advance in knowledge on the molecular basis of human disease. Due to the applicability and importance of this technology in basic research, it has quickly migrated to the clinical setting. NGS enables clinicians to make improved diagnostic and treatment decisions, which ultimately may influence precision medicine. (REV INVES CLIN. 2018;70:153-7)

Key words: Next-generation sequencing. Clinical diagnostics. Clinical genomics.

INTRODUCTION

Next-generation sequencing (NGS) technologies are being widely adopted in the clinical setting providing clinicians with improved tools to understand the complex mechanisms underlying patient's disease and facilitating further advances in early diagnostics, prediction of disease risk, and therapeutic decision making in a clear path toward precision medicine¹.

The main applications of NGS in clinical diagnostics include the detection of mutations, splice variants, structural alterations, gene fusions, single nucleotide

variations, small INDELs, copy number variations, comparison of expression profiles, and genome-wide association studies. The most used techniques include, but are not restricted to, whole-genome sequencing (WGS), whole-exome sequencing (WES), transcriptome sequencing, and a variety of targeted sequencing panels designed to analyze specific sets of genes, allowing greater sequencing depth and coverage and increased analytical sensitivity and specificity at a reduced cost (Table 1)². This technology has been widely adopted in a number of clinical and research fields including oncology, prenatal diagnosis, and microbiology among others, particularly because

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most of the NGS protocols can be applied with the established sample collection techniques from blood, buccal swab, saliva, skin punch, and tumors biopsies or formalin-fixed, paraffin-embedded tissue (Fig. 1).

CLINICAL APPLICATIONS OF NGS

The oncology field has benefited the most from NGS. The number of genomic alterations found by WGS and WES profiling from a variety of tumor samples and their corresponding normal tissues have scaled up in recent years. Furthermore, the use of NGS for hereditary cancer risk assessment is becoming a reality in cancer genetics, such that different leading cancer centers members of the National Comprehensive Cancer Network (NCCN) now include the use of multi-gene testing for familial breast and ovarian cancer risk assessment³. Recent technical advances allow the clinical scientist to perform genomic profiling on samples derived from small amounts of paraffin-embedded tumor tissue, single cells, or tumor-derived cell-free DNA from plasma with high sensitivity and specificity⁴. NGS can also help to elucidate the mechanisms of drug resistance and guide subsequent treatment, by identifying, for example, new mutations in biopsies at disease relapse, but the power of NGS in the clinical setting seems to focus on the gene panels, where oncologists may obtain data from a selected list of genes relevant for the specific type of tumor allowing a deeper coverage at reduced cost⁵. A variety of panels are available for different tumors including breast, colorectal, and familial cancer, among many others, and the U.S. Food and Drug Administration (FDA) has already approved some of these detection panels (Table 1). In this context, the use of NGS in the clinical setting represents a unique opportunity to explore the relationship between the defined molecular abnormalities and response to drugs with targeted mechanisms of action⁶. Considering these challenges, a number of multicenter Umbrella and Basket trials have been initiated. In the Umbrella trials, patients with a single tumor type are enrolled and treated depending on the different molecular biomarkers. On the other hand, in the Basket trials, patients with different tumor types are selected based on the same biomarker profile and receive a single treatment. The clinical trial NCI-MATCH is a precision medicine cancer treatment where patients are assigned to receive therapy based on the genetic changes found in their

tumors through genomic sequencing and other tests⁶. It is important to mention that these programs have implemented the integration of “Genomic/Tumor Boards”, where the attending clinician and a panel of experts including oncologists, geneticists, bioinformaticians, and other experts discuss the molecular findings and make clinical recommendations to each patient.

In other areas, whole-genome and exome sequencing of fetal material derived from amniocytes or chorionic villi are being already performed in specialized centers, though it is not a routine practice. In this area, several factors must be considered including gestational age, sample type, and maternal cells, which may lead to DNA contamination. Nevertheless, prenatal genomic sequencing offers an opportunity for early intervention and a potentially higher likelihood of effective treatment⁷. Furthermore, precisely due to the greater sensitivity of NGS and the realization that fetal DNA is circulating in the mother's serum, we now see the emergence of the field of non-invasive prenatal testing as a more convenient and safe alternative to this invasive tests⁸.

On the other hand, NGS is also being used in medical microbiology for molecular diagnostics, outbreak monitoring, characterization and surveillance of pathogens, as well as detection of novel resistance genes. The most popular application is metagenomics, in which, for example, sequencing of the 16S ribosomal subunit of bacteria may help in the identification of a diversity of species in different samples⁹. In addition, sequencing whole bacterial DNA or RNA from clinical samples by shotgun metagenomics may allow detecting the presence of pathogen-associated virulence and resistance genes.

In recent years, many groups have used NGS (WGS or WES) to identify disease-associated variants in a growing number of conditions, including neurological diseases such as spinocerebellar ataxia, Charcot-Marie-Tooth disease, hereditary spastic paraplegia, and amyotrophic lateral sclerosis, among others, providing an opportunity to discover new therapeutic strategies for these severe diseases. Another particular NGS application is the human leukocyte antigen (HLA) typing, which yields a comprehensive profiling of the HLA loci for applications in the field of organ transplantation. In fact, it has been recently adopted by the

Figure 1. Applications of next-generation sequencing (NGS) in the clinical setting. **(A)** Applications available for the clinicians depend on the initial nucleic acid type, DNA (genomics), or RNA (transcriptomics). Samples can be obtained from different sources and tissues applying the already established protocols and analyzed in a global (whole-genome sequencing or whole-exome sequencing) manner or using specific gene panels. **(B)** The NGS workflow starts with the sample selection, purification, and quantification of the chosen nucleic acid and the selection of the appropriate library method, followed by sequencing and data analysis. Integration of a genomic board is crucial to review all the data and provides clinical recommendations that are delivered by the attending clinician.

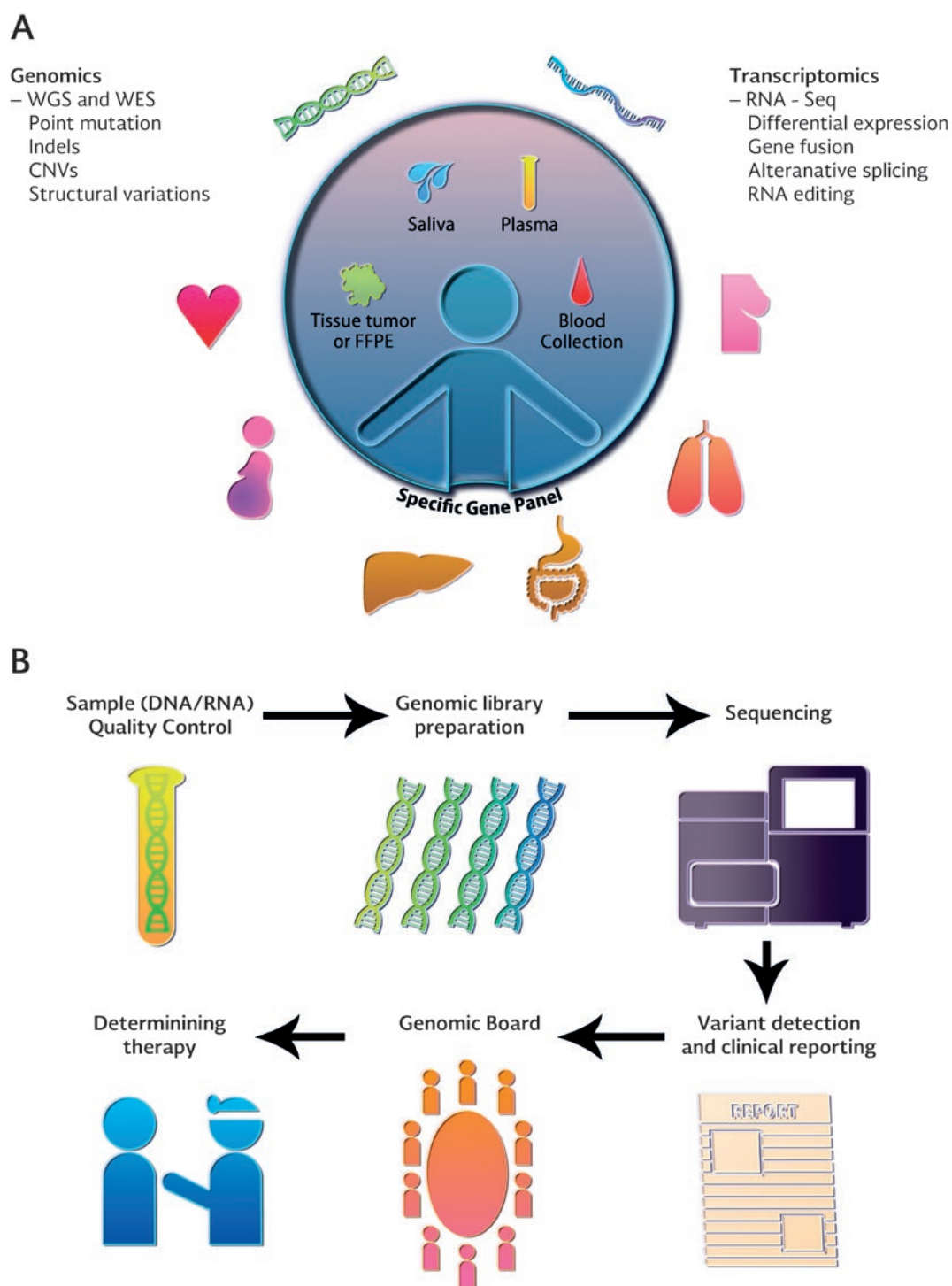


Table 1. Brief list of next-generation sequencing panels for clinical use.

Company	Product	Number of analyzed genes	Target	FDA approved
Cancer				
Thermo Fisher	Oncomine Dx™	52	Hotspots, SNVs, INDELs, CNVs, and gene fusions	Yes
Illumina	TruSight® Cancer Panel	94	1700 exons and 284 SNPs	—
Foundation medicine	FoundationOne®	315	300 cancer—related genes, including select introns from more than 25 genes often rearranged or altered in solid tumors	Yes
Foundation medicine	FoundationOne® Heme	405	Gene fusions	—
Foundation medicine	Foundation Focus™ CDxBRCA	2	BRCA1 and BRCA2 mutations	Yes
Myriad Genetics	BRCA Analysis	2	Gene mutations	Yes
Illumina	MiSeqDx Cystic Fibrosis Clinical Sequencing Assay	1	Intron/exon of the CFTR gene, mutations, and INDELs	Yes
Illumina	Colorectal and Endometrial Cancers	19	Diagnostic/surveillance of Lynch syndrome—like sporadic	—
Illumina	Extended RAS Panel	2	12 codons in KRAS/NRAS, 56 activating mutations	Yes
Ion Torrent	Ion Ampliseq hotspot	46	Tumors suppressor genes and oncogenes	—
Illumina	TruSeq Amplicon	48	48 genes are targeted with 212 amplicons, oncogenes	—
Ion Torrent	Various solid Tumor samples	409	All exons of key tumors suppressor genes and oncogenes	—
Immunology				
Illumina	TruSight HLA Sequencing	HLA	11 HLA loci	—
Reproductive Health				
Illumina	VeriSeq PGS		24 chromosomes for selection of embryos most likely to be euploid	—
Genetic Health				
Illumina	TruSight Inherited Disease	552	Mutation, intron—exon	—
Illumina	TruSight One Sequencing	4813	Coding regions of genes with associated clinical phenotypes	—
Cardiomyopathy				
Illumina	TruSight Cardio Sequencing	174	Cardiomyopathies, arrhythmias, aortopathies, and more	—

China Marrow Donor Program (The Data Bank of Chinese Hematopoietic Stem Cell Donors) for ultra-large-scale genotyping of donors indicating that NGS is cost-effective and a valuable tool¹⁰.

BIOINFORMATICS

A great challenge in NGS is bioinformatics analysis and data interpretation. Current NGS instruments can

generate up to almost 1 terabase of information per run (10^{12} bp). These billions of sequences are then aligned and compared to a reference genome to identify variants or mutations¹¹. These analyses require a solid bioinformatics infrastructure and personnel to handle the impressive amount of data generated for storage and analysis. One advantage of the commercial gene panels is that in many cases, they include access to online programs that perform the analysis and generate a clinical report. For example, SOPHiA DDM, a software platform from SOPHiA Genetics, detects, annotates, and pre-classifies raw genomic data to help clinicians to reach a better and faster diagnosis of patients across a broad range of diseases.

NGS PLATFORMS AVAILABLE

Among the several NGS systems currently available, including Pacific Biosciences, Oxford Nanopore, Ion Torrent (Thermo Fisher Scientific), and Illumina, the last two systems are the most employed in the clinical setting. The Ion Torrent semiconductor-based sequencing technology has gained considerable acceptance and has recently launched the Global Ion Torrent Developers Alliance Program that provides the users with flexible options to access Ion Torrent sequencing systems and the Ion AmpliSeq and OncoPrint reagents. The Ion Torrent OncoPrint Dx Target Test is the first FDA-approved test that can detect multiple gene mutations in patients with metastatic non-small cell lung carcinoma. On the other hand, Illumina sequencing by synthesis chemistry has become the most widely used technology. The MiSeqDx System is the first FDA-cleared NGS platform for *in vitro* diagnostic testing, and a collection of panels for multiple diseases and the options for WGS or WES are available on all their platforms. In all cases, the instruments provide precise and reliable screening for diagnostic testing, with the speed and accuracy necessary for early disease detection, prognosis, or personalized treatment (Table 1). On a final note, the use of WES, WGS, or any type of sequencing has raised legal and ethical issues such as how and when to disclose incidental findings to the patient or study participant, and the data protection and access are still a concern.

CONCLUSION

The role of NGS in molecular diagnostics, disease prognosis, and pharmacologic therapy will increase further during the coming years. The advancement in sequencing technologies and the drop in sequencing costs will soon enable researchers to study genomes with greater coverage and depth and, more important, to follow an integrative genomics approach to associate genomic sequencing with transcriptomics and epigenomics data to identify clinically useful biomarkers for direct application in precision medicine.

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