NGS technology: Trends and clinical applications

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DNA sequencing using next-generation technologies has become established in several clinical areas over the past decade and is rapidly gaining popularity in many others. Reproductive health, oncology, Mendelian diseases, complex diseases, and infectious diseases are key areas where next-generation sequencing (NGS) has a significant presence or is expected to establish itself in the coming years.

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As the Human Genome Project was coming to an end in 2003—two years ahead of schedule—new technological developments sparked a second revolution in the study of genomes. These so-called next-generation sequencing (NGS) techniques use massively parallel sequencing to decode large areas of the genome, orders of magnitude faster than Sanger sequencing, which had been key to the success of the Human Genome Project (1).

NGS technologies developed in the decade that followed fall into two categories: those that use clonal amplification (sometimes known as second-generation sequencing) and single-molecule techniques (third generation). These innovations helped drive down the cost per nucleotide to the extent that it opened up NGS to a wide range of clinical applications.

Recognizing the potential of NGS not only to identify underlying causes of disease, but also predict responses to interventions and determine individuals at risk, the 100,000 Genomes Project was initiated in 2012. Thanks to the continued evolution of NGS technologies, this project has now accomplished its aim of sequencing 100,000 whole genomes of individuals with rare diseases, their families, and individuals with some cancers—yielding a wealth of new diagnostic information that directly benefits patients (2). In recent years, third-generation instruments have become increasingly miniaturized with the development of palm-sized sequencers suitable for use in the field.

Despite the enormous advances in sequencing technology, however, significant gaps remain in knowledge of the human genome. In November 2018, for example, a team of scientists reported that whole genome sequencing of 910 individuals revealed a large amount of DNA sequences not present in the reference genome. These sequences represent almost 10% of the entire genome (296,485,284 base pairs) and included 315 protein-coding genes (3)—an eye-opening reminder of the uncharted territory that still exists. While NGS is still an emerging technology in clinical settings, it has rapidly gained acceptance in several areas. This white paper discusses five key fields where NGS is already established as a valuable enabler of precision healthcare: reproductive health, oncology, Mendelian diseases, complex diseases, and infectious diseases (4).

Reproductive Health

Background

In 2017, reproductive health was the largest clinical market for NGS testing by revenue (4). Genetic screening in reproductive health can involve testing at various stages of development:

- before implantation of the embryo
- during pregnancy
- shortly after birth

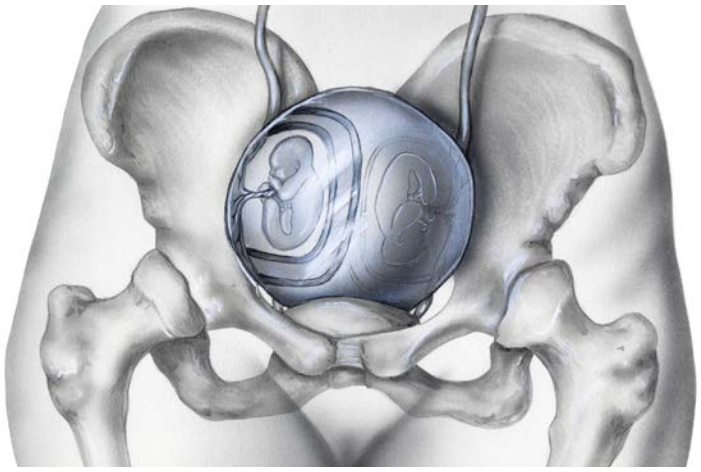

Fig. 1. Non-invasive prenatal testing (NIPT) is a key focus of NGS.

A focus for the NGS market within reproductive health is non-invasive prenatal testing (NIPT). During pregnancy, many parents have their unborn child tested for conditions such as Down Syndrome and other forms of chromosomal aneuploidy, an abnormal number of chromosomes.

Conventional prenatal genetic testing for chromosomal aneuploidy involves amniocentesis (collecting fetal cells from the amniotic fluid) and chorionic villus sampling (collecting placental tissue). Although reliable, these methods carry a risk of miscarriage, which might be as high as 1 to 2% (4).

An NGS-based approach

The NGS approach for NIPT involves analyzing cell-free fetal DNA. During pregnancy, a small amount of fetal DNA originating from the placenta ends up in the maternal blood circulation. As this DNA is more fragmented than most maternal DNA in the bloodstream, size selection enables NGS assays to distinguish cell-free fetal DNA from most maternal DNA (5).

The central benefit of this approach is that it is non-invasive, relying on a maternal blood sample and avoiding the need to access the womb for sample collection. As a result, there is a lower risk of miscarriage associated with this technique. This key benefit has led to a surge in the use of NGS for prenatal testing since its initial development in 2011, with more than a dozen commercial tests currently available for this application.

Due to the low quality and quantity of cell-free fetal DNA available for analysis, this type of test was initially only able to detect the largest genomic abnormalities, such as trisomy 13, 18, and 21. However, as the technology has evolved, it has also become possible to study sub-chromosomal abnormalities, such as microdeletions.

Oncology

Background

The field of cancer diagnostics has long been a large market for clinical NGS-based tests. Cancer is one of the leading causes of death worldwide, with an estimated 17 million new cases diagnosed annually. Due to global increases in life expectancy, the incidence of cancer is likely to rise by around 62% by 2030 (6).

Furthermore, the link between cancer and genetics is well-established. Cancer can be defined by abnormal tissue growth caused by mutations in the genes that regulate cell growth over a person's lifetime. Inherited forms of several genes, such as BRCA1/2, also play a role in the risk of developing certain cancers (7).

The most common way to classify and determine treatment for cancers is by the tissue in which the cancer originates. However, there is increasing understanding that, in many cancers, determining effective treatment depends more on genetic factors, further highlighting the importance of genetic testing for cancer diagnosis and screening.

Fig.2. The efficacy of targeted therapies depends on the molecular profile of the tumor.

NGS in cancer diagnosis

Whether or not DNA sequencing of tumor cells is a suitable approach for any given patient depends on the treatment options available for that specific cancer. Many cancers have targeted therapies, whose efficacy depends on the molecular profile of the tumor. In these cases, a detailed understanding of a tumor's genetic make-up can provide information on whether a therapy, which might have high costs and severe side effects, is advisable.

In cases where these targeted therapies are a viable option, sequencing on a biopsy or sample removed during surgery can help identify the precise genetic origin. There is also a growing trend towards liquid biopsies—samples of blood or urine—from which cell-free DNA (cfDNA) is isolated, thereby avoiding the need for invasive procedures.

The number of nucleotides analyzed in a clinical test varies; commonly offered approaches include:

- whole genome sequencing
- whole exome sequencing
- targeted sequencing

Targeted sequencing assays, which typically look at tens to hundreds of genes, are currently the most popular approach. These NGS panels target a select range of genes most likely to contain cancer-causing mutations and come at a substantially lower cost than, for example, whole genome sequencing (7). However, this balance might shift as sequencing costs decrease and more comprehensive sequencing becomes cost-effective.

NGS in cancer screening

In groups of people at higher risk of cancer, genetic testing for cancer predisposition can play an important role in reducing cancer mortality through prevention. Although cancer is generally not inheritable, inherited genetic variants can affect the risk of certain common cancers, such as breast, ovarian, colorectal, and prostate (8).

For people with a family history of these cancers, NGS-based testing is well-suited to identifying any inherited harmful variants, particularly when they have already been identified in relatives with cancer.

As preventive cancer screening can benefit a significant proportion of the population, there is considerable growth potential for NGS assays. A key challenge is to bring down the cost of preventative screening assays such that NGS methods become an attractive option in cancer diagnostics for groups with an elevated, but low absolute risk of developing cancer.

> ***Initiative: Cancer Moon Shots Program***
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> *One of the projects aiming to improve diagnosis and treatment of cancer is the MD Anderson Cancer Center's "Cancer Moon Shots" program (mdanderson.org/cancermoonshots.html). Its mission is to tackle cancer through "innovation, scale and collaboration". It has funding of 1.8 billion USD over a period of 10 years, and treats more than 100,000 patients annually in over 1,200 clinical trials. The program uses NGS alongside other techniques for sequencing to study eight common cancers.*

Mendelian diseases

Background

Mendelian diseases are a diverse range of heritable conditions, so called because they follow the pattern of inheritance as originally set out by Gregor Mendel. These diseases originate from single-gene variants, for example single nucleotide polymorphisms (SNPs) and indels, that parents can pass on to their offspring, and can be dominant or recessive in nature.

An estimate of the total number of Mendelian disorders is in the region of 6,000. Some of these disorders, such as cystic fibrosis, are understood well. However, most conditions that show Mendelian inheritance are rare and, for many of these, a genetic origin has not yet been identified (4).

Conventional diagnosis

This variability and rarity of Mendelian, single-gene disorders make many of these conditions difficult to diagnose. Symptoms can range from cardiomyopathy, deafness, and retinitis pigmentosa to cognitive impairment.

Diagnosis often consists of a lengthy process of different tests carried out sequentially, aimed to exclude potential causes one by one. Sanger sequencing is a commonly used molecular diagnostics method for this due to its accuracy and, when studying small genomic regions, low cost.

For large regions of the genome spanning multiple genes, however, the cost of Sanger sequencing can increase dramatically as it offers limited opportunities for multiplexing. Diagnosis of several common conditions, such as deafness, blindness, mitochondrial disease, and movement disorders is not cost-effective using Sanger sequencing (9).

The value of NGS

The large number of genes that could be responsible for any given Mendelian disease present a key opportunity for NGS-based assays. NGS can be used to efficiently analyze multiple genes simultaneously, making it well-suited to searching for rare variants in a pool of many genes.

As a result, NGS assays provide the opportunity to sequence a higher number of targets than with Sanger sequencing, with little difference in costs. Compared to sequential molecular tests, the all-in-one approach offered by NGS also reduces the time to find the cause of a condition, contributing substantially to the well-being of patients and their relatives (10).

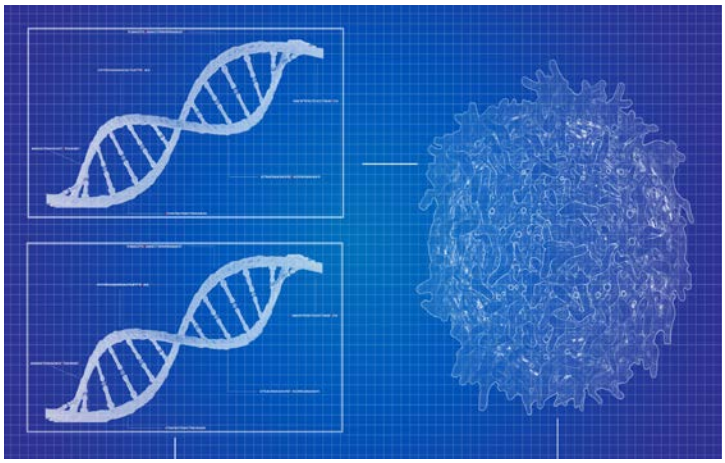

Fig.3. The large number of genes that could be responsible for any given Mendelian disease present a key opportunity for NGS-based assays .

Even though NGS is already applied successfully in the clinic, demonstrating its efficacy and benefits is an ongoing challenge for expanding market share. However, as uptake increases, the improved data availability can provide a new boost to the field, particularly as it becomes possible to link ever rarer conditions to genetic abnormalities, paving the way for successful treatment.

> ***Initiative: Human Immunology Project Consortium***
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> *Established in 2010, the Human Immunology Project Consortium (HIPC) is one of a growing number of initiatives bringing together molecular diagnostics data from multiple sources and making it freely available to the public. It was set up by a division of the National Institutes of Health (NIH) and studies "well-characterized human cohorts using a variety of modern analytic tools to provide a comprehensive understanding of the human immune system and its regulation." (immuneprofiling.org/)*

Microbiology and infectious diseases

Background

Identification of microorganisms is a key challenge in many clinical settings. Obtaining precise information about the pathogen responsible for food-borne or hospital-acquired infections plays an essential role in guiding treatment.

Conventional methods for pathogen identification are varied and might depend on symptoms or the type of pathogen. These methods include phenotypic assays as well as genetic techniques such as polymerase chain reaction (PCR) or Sanger sequencing. When analyzing bacterial DNA with Sanger or PCR, results are accurate and can sometimes provide results within hours (13,14).

However, there are also limitations associated with these conventional techniques. PCR and Sanger sequencing require a certain level of knowledge about the microorganism to identify areas of the genome for targeted investigation. This selection creates so-called 'biased assays', in which a pathogen is less likely to be detected if they are not optimized for that specific pathogen (14).

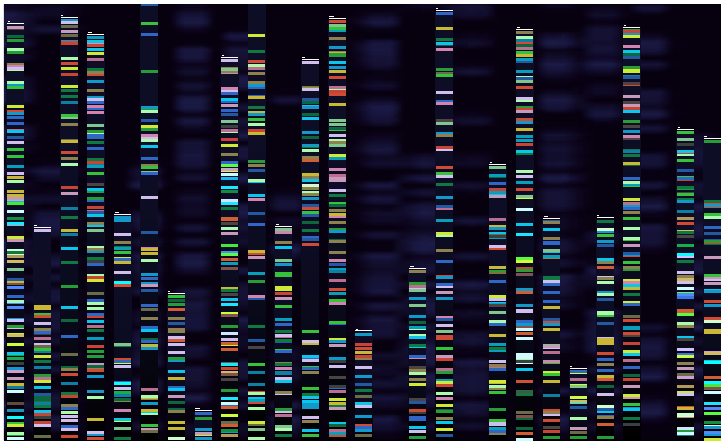

Fig.4. NGS-based assays can provide the multiplexing capacity to sequence an entire bacterial genome in a day.

Identifying pathogens with NGS

NGS-based assays can provide the multiplexing capacity to sequence an entire bacterial genome in a day, overcoming the limitations of PCR and Sanger sequencing, and enabling rapid diagnosis. Whole genome sequencing through NGS also avoids the need to select targets or risk running biased assays. Unbiased NGS assays have the benefit of avoiding false negatives that might arise when primers fail to bind due to mutations at the binding site. They also help in detecting co-infections where other assays might only detect one species.

Another strength of NGS approaches, compared to phenotypic assays, is that they do not require culture before analysis. Isolation and culture present a problem for organisms that are difficult to culture in the laboratory. Examples include *Mycobacterium tuberculosis* and *Mycobacterium leprae*. In these situations, NGS can make detection easier and faster than when using conventional methods (14).

NGS-based assays provide doctors with detailed information about a pathogen faster than conventional methods, and so accelerate the ability to recommend targeted treatments for infections and prevent or limit disease outbreaks. Using NGS for pathogen sequencing can also help reduce the use of antibiotics as a first-line treatment for an unspecified infection, thereby reducing the likelihood of pathogens developing antibiotic resistance.

The future of NGS in clinical applications

The five key areas discussed, reproductive health, oncology, Mendelian diseases, complex diseases, and infectious diseases, all use NGS in clinical practice, though the market share compared to competitive technologies varies. In the next few years, NGS is likely to diversify into other clinical areas as sequencing costs decrease and it becomes increasingly recognized across disciplines (4).

In addition, there is a range of applications within these key areas where NGS is making inroads and is likely to become an established method in the coming years. Examples that show substantial promise include liquid biopsies for early detection and monitoring of cancer, and diversification of the reproductive health market.

Liquid biopsy sequencing in oncology

In 2017, oncology was the second biggest clinical market for NGS-based assays (after reproductive health). It is expected to be the largest by 2022, with the analysis of liquid biopsies contributing a large part of this growth. Many tumors shed fragmented DNA at rates far above healthy tissue, meaning that affected individuals can have cfDNA levels up to 50 times higher than healthy individuals. The typical length of this circulating tumor DNA (ctDNA) is several hundreds of nucleotides, which makes it suitable for analysis with NGS (15).

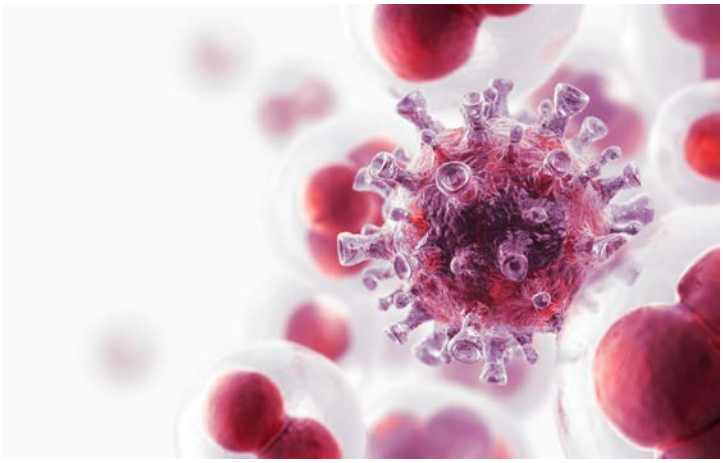

Fig.5. NGS is likely to become an established method for early cancer detection and monitoring using liquid biopsies in the coming years.

Liquid biopsies for cfDNA screening have shown potential for both early detection and monitoring of cancer. Their key benefits—very low risk of false positives and the ability to detect millimeter-sized tumors—make it a viable alternative to techniques such as MRI and CT. Sequencing can also detect new emerging mutations in ctDNA, enabling the adjustment of treatment where necessary (4).

The central challenge for growth in this application is in improving the sensitivity of assays. After tumor removal, levels of circulating cfDNA drop rapidly, but mutation levels as low as 0.1% could still indicate residual tumor presence. These low-level mutations, combined with the possibility of PCR-induced errors, mean that improving sensitivity of NGS-assays for liquid biopsies will be instrumental in expanding market share in oncology.

Initiatives: GRAIL, Freenome

Several projects employ the use of NGS to analyze liquid biopsies with the aim of developing blood tests for early cancer detection and treatment stratification. GRAIL (grail.com) builds "intelligent models to identify clinically actionable information from vast amounts of tumor genome data obtained through high-intensity sequencing combined with modern data science." Freenome's approach (freenome.com) goes even further, using artificial intelligence to look "beyond mutations to detect the body's own early-warning signs for cancer, including real-time changes in gene expression, immune activity, and cancer-associated proteins."

Diversifying the reproductive health market

NIPT made up around 95% of the reproductive health market for NGS in 2017. Although substantial growth is expected, other applications such as carrier screening, preimplantation genetic screening in IVF, and newborn screening are also expected to contribute to the growth of this market in the coming years (4).

Screening embryos for genetic disorders can help improve IVF success rates by detecting Down syndrome and other forms of chromosomal aneuploidy. NGS testing can also confirm whether a genetic defect from a parent is present in the embryo (16).

In newborn screening, the heel prick blood spot test is standard in many countries (17). NGS can play a role in addition to this test, providing more detailed information in case of a positive result in routine screening.

Carrier screening involves testing adults, typically before trying to conceive, to discover whether they carry defects that could be passed to any offspring. NGS can help parents make informed decisions regarding family planning and potential further testing.

Conclusion

In research, NGS has been the method of choice in many sequencing applications for over a decade. Clinical NGS follows this trend with substantial growth in the molecular diagnostics markets, as discussed in this white paper, as well as in other markets.

As shown by the 100,000 Genomes Project, genomic medicine is already helping to transform healthcare services. This project made real progress in addressing the lack of sufficient quality DNA for whole genome sequencing, a gap that had made the application of genomics in cancer diagnosis and treatment problematic. Thanks to the application of NGS in these areas, many patients are finally receiving diagnoses for syndromes that were simply previously unidentified, providing avenues for support and understanding, as well as opening the potential for highly tailored treatments (18).

The future of NGS in the clinic will be one of continued growth in existing and new applications as costs decrease, more NGS-based assays become available, and the added value of NGS data over conventional tests becomes increasingly recognized.

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