

# Next-Generation Sequencing in Pathology: Enabling Personalized Medicine

The material is intended to provide health care providers (HCPs) with basic information on next-generation sequencing and is for general educational purposes only. The guide is not intended to be used to substitute for the exercise of the HCP's professional judgment in providing professional services.

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# Molecular Pathology and Genomics

The fast-paced development of genomic sequencing technologies is revolutionizing our understanding of the complexities of cancer. The number of variants identified as being associated with cancer risk onset and progression is rapidly growing. Several well-characterized genes, erbB2 receptor tyrosine kinase 2 (*HER2*), epidermal growth factor receptor (*EGFR*), *BRAF*, and RAS sarcoma genes (*KRAS*, *NRAS*), are now incorporated into molecular cancer testing procedures.<sup>1</sup> Next-generation sequencing (NGS) is enabling the investigation of mutations associated with cancer risk and prognosis.<sup>2</sup> Genomics is revolutionizing the discovery and investigation of novel drug targets that are leading to earlier diagnosis and more targeted cancer therapies. Today, several companion diagnostic devices have been cleared or approved (**Table 1**).<sup>3</sup>

Cancer Type	Gene	Variant(s)	Targeted Therapy
Colorectal	<i>KRAS</i>	Exons 2, 3, 4	Vectibix (panitumumab)
		Codons 12, 13	Erbitux (cetuximab)
	<i>NRAS</i>	Exons 2, 3, 4	Vectibix (panitumumab)
Non-small cell lung cancer	<i>EGFR</i>	Exon 19 deletions Exon 21 (L858R) substitution	Tarceva (erlotinib) Gilotrif (afatinib) Iressa (gefitinib)
		T790M	Tagrisso (osimertinib)
Melanoma	<i>BRAF</i>	V600E	Mekinist (trametinib) Zelboraf (vemurafenib) Tafinlar (dabrafenib)
		V600K	Mekinist (trametinib)
Breast	<i>erbB2 (HER2)</i>	Amplification	Herceptin (trastuzumab)

**Table 1.** A few examples of gene variants that are associated with cancer that have targeted therapies.<sup>3</sup>

## Next-Generation Sequencing

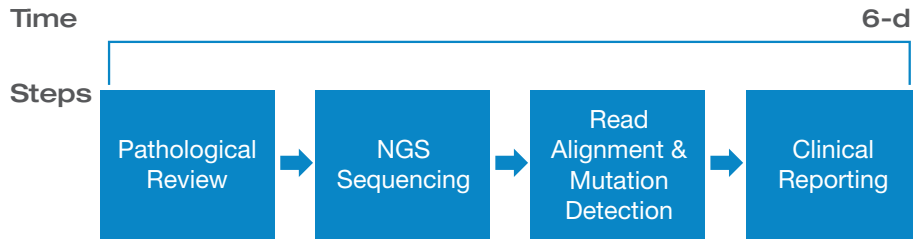
NGS offers several advantages over traditional, more labor-intensive approaches such as Sanger sequencing, polymerase chain reaction (PCR) testing, fluorescence in situ hybridization (FISH), and single-gene assays. Given the narrow focus of these molecular methods, additional testing may be required. Such sequential testing is dependent on tissue availability and may require additional biopsy procedures. In contrast, by analyzing multiple genes and multiple samples in a single experiment, NGS can reduce the time to meaningful results.

Several organizations, including the Association for Molecular Pathology (AMP), the American College of Medical Genetics and Genomics (ACMG), the College of American Pathologists (CAP), and the US Food and Drug Administration (FDA), recently published guidelines, guidance documents, or recommendations for sequencing.<sup>4-6</sup> NGS is changing the paradigm in molecular pathology, moving from clinical diagnosis alone to providing evidence supporting treatment recommendations.

## NGS Workflow in Cancer

In principle, the concept behind NGS technology is similar to Sanger sequencing. The breakthrough innovation is that instead of sequencing a single DNA fragment, NGS extends this process across millions of fragments in a massively parallel fashion. This method is highly scalable and can be applied to a subset of key genes (as seen in targeted panels) or to thousands of genes simultaneously (whole-exome sequencing [WES] or whole-genome sequencing [WGS]). Prior to testing, a pathologist examines the tissue specimen to confirm the presence and percentage of tumor cells, their viability, and cellularity. DNA is extracted from the tumor specimen, nucleotide sequence is determined, detected variants are interpreted, and the clinical significance is reported. This process or

turnaround time (TAT) takes approximately 6 days (**Figure 1**).<sup>7</sup> Recently, the AMP and the CAP jointly published recommendations for analytical validation of NGS-based oncology gene panel testing to improve the quality of sequencing and thus provide better care for patients with cancer.<sup>8</sup>



**Figure 1.** Clinical NGS testing in molecular pathology. Adapted from Macmillan Publishers Ltd: Frampton GM, et al. *Nature*. 2013;31(11):1023-1033.<sup>9</sup>

## NGS Applications

Compared with traditional methods, NGS offers advantages in accuracy, sensitivity, and speed that can make a significant impact on the field of molecular pathology. NGS can be used to study many types of variations, including structural changes, fusions, single nucleotide variants (SNVs), small indels, gene expression, and DNA methylation.<sup>10-13</sup> Various NGS applications are available for testing cancer samples: WGS, WES, and targeted panel sequencing, each offering different advantages (**Table 2**).<sup>14,15</sup>

NGS Applications	Description	Advantages	Disadvantages
<b>WGS</b>	Determines the DNA sequence of an individual's genome	<ul style="list-style-type: none"> <li>Provides genetic information of coding and noncoding regions</li> <li>Provides a comprehensive approach</li> <li>Majority of known pathological abnormalities are in the exome</li> </ul>	<ul style="list-style-type: none"> <li>Data may be more difficult to interpret</li> <li>Challenges with incidental findings</li> </ul>
<b>WES</b>	Determines the DNA sequence of the protein coding (expressed) regions of an individual's genome	<ul style="list-style-type: none"> <li>Majority of known pathological abnormalities are in the exome</li> <li>Functional consequences of variants are more easily understood</li> </ul>	<ul style="list-style-type: none"> <li>Misses variants in noncoding regions and some structural variants</li> <li>Challenges with incidental findings</li> </ul>
<b>Targeted</b>	Determines the DNA sequence of specific genes or gene regions	<ul style="list-style-type: none"> <li>Usually less expensive than WGS/WES</li> <li>Focused on specific genes, so data interpretation is easier</li> <li>Little concern regarding incidental findings</li> <li>Can optimize gene panel to capture difficult regions</li> </ul>	<ul style="list-style-type: none"> <li>Does not provide information on regions outside the gene panel</li> </ul>

**Table 2.** Comparison of NGS applications used in oncology.<sup>14</sup>

## Whole-genome sequencing

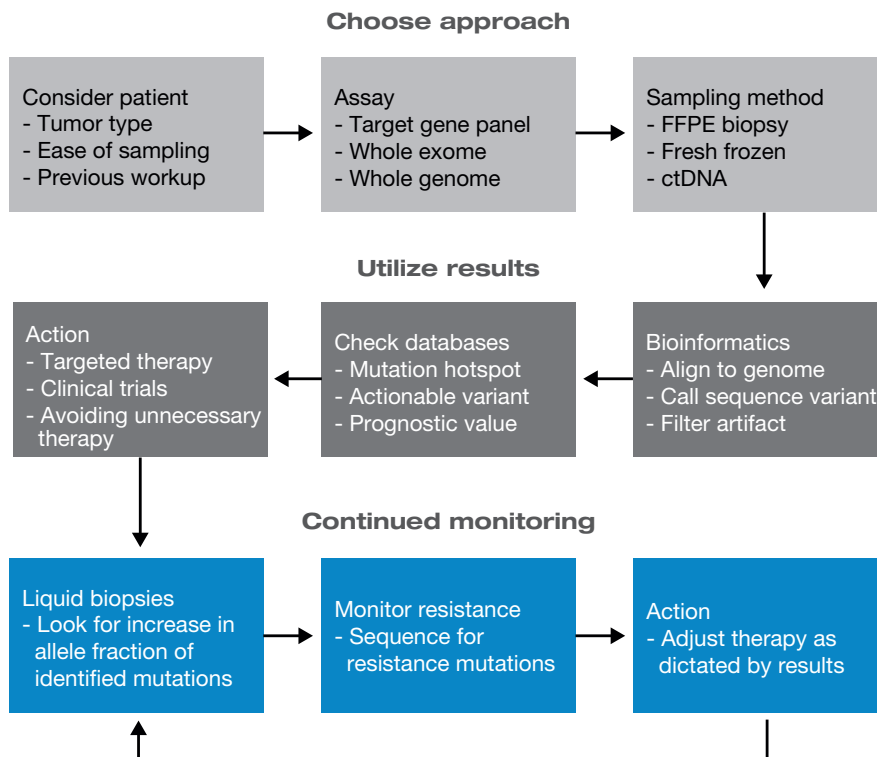
WGS can detect variants across the genome, both coding and noncoding regions, enabling the discovery of novel cancer-associated variants, such as SNVs, copy number changes, and structural variants. Moreover, by comparing tumor and normal DNA, WGS can provide a comprehensive view of changes to a specific tumor sample. However, the large amount of data generated can be more difficult to interpret, and challenges still exist around the interpretation of incidental findings.<sup>16</sup>

## Whole-exome sequencing

WES focuses on the coding regions, approximately 1% of the total genome.<sup>17,18</sup> Up to 85% of cancer variants are found within exons and therefore, may be easier to interpret than intronic variants. WES allows for deeper coverage of the genome, supporting identification of low frequency variants within heterogeneous tumor samples.<sup>11</sup> Therefore, WES may sometimes be a more cost-effective approach than WGS.

## Targeted panels

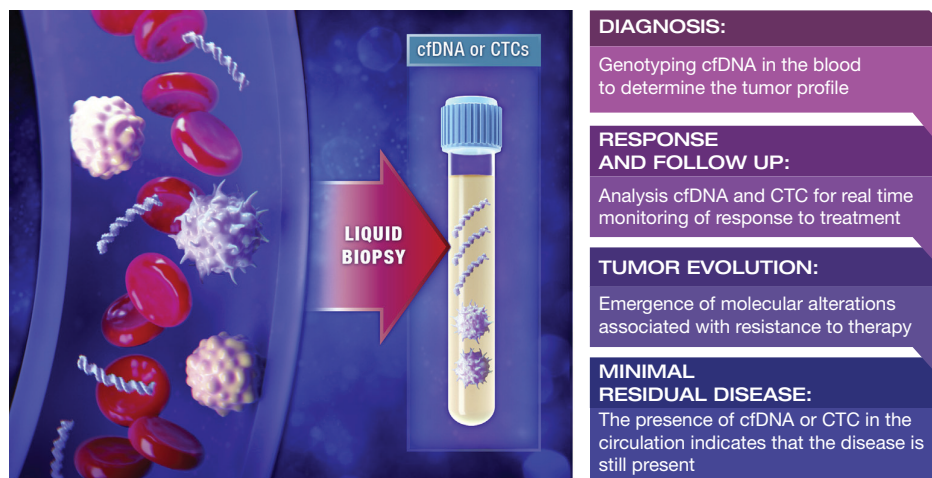
Targeted sequencing, the most frequently used NGS method in molecular pathology, focuses on specific genes or gene regions commonly mutated in cancer (**Figure 2**).<sup>14</sup> This approach offers some benefits over WGS and WES, including lower cost (depending on panel size), fewer incidental findings resulting in easier data interpretation, and gene panel optimization for capturing regions that can be difficult to sequence.



**Figure 2.** Approach for using NGS in the diagnosis and monitoring of disease in patients with cancer. Sequencing results are interpreted into clinically actionable information, therapy is delivered, and disease is continually monitored. Reprinted from Gagan J, Van Allen EM. *Genome Med.* 2015;7(1):80. Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>).<sup>17</sup>

## NGS for monitoring disease using circulating tumor DNA (ctDNA)

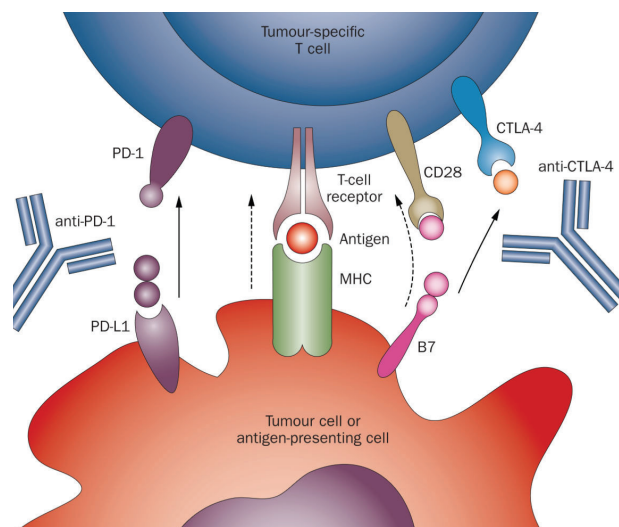
NGS is being used to monitor disease by analyzing the circulating tumor DNA (ctDNA), and in some cases the circulating tumor cells (CTCs), that can be found in the blood. ctDNA is derived from the rapid turnover of cancer cells due to apoptosis and necrosis, resulting in the constant release of tumor-derived nucleic acids into the circulation.<sup>19</sup> Applications of ctDNA include genomic profiling of tumors, monitoring of therapeutic response, detection of mutations associated with treatment resistance, and tracking of minimal residual disease (MRD) following treatment (**Figure 3**).<sup>20</sup> The preferred source of ctDNA is plasma, given its lower background level of wild-type DNA. However, ctDNA has also been found in other biological fluids, including urine, saliva, pleural effusions, and cerebrospinal fluid.<sup>21-24</sup> Although tissue biopsy remains the standard of care, it reflects only a single point in time from a single tumor site. The diversity of molecular genetic information found within each tumor and metastases makes tissue biopsy an inadequate method for comprehensive longitudinal genomic characterization.<sup>25</sup> Continued refinement of ctDNA technology has the potential to revolutionize the way cancer is identified and treated, leading to earlier diagnoses, better survival rates, and improved quality of life.



**Figure 3.** Clinical applications using cell-free-DNA (cfDNA) and CTCs detected in blood, the liquid biopsy. Reprinted from Bardelli A, Pantel K. *Cancer Cell*. 2017;31(2):172-179, copyright 2017, with permission from Elsevier.<sup>20</sup>

## NGS for identifying biomarkers for immunotherapy using checkpoint inhibitors

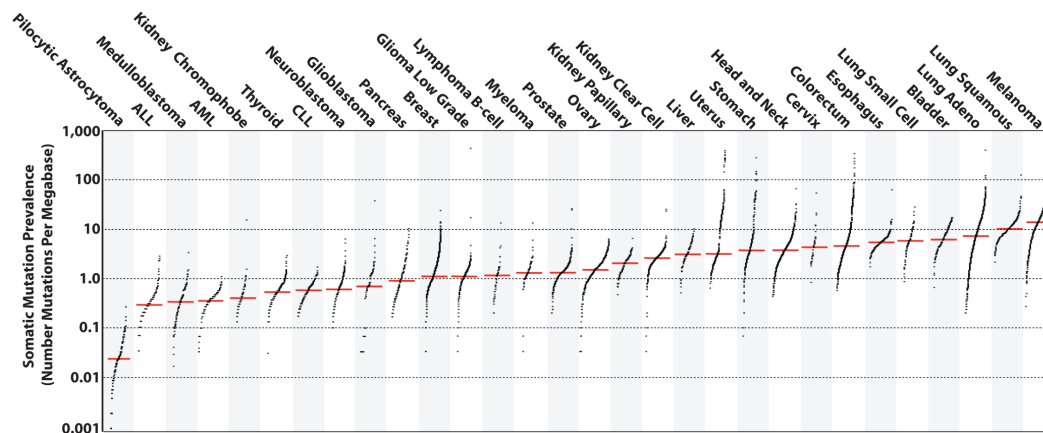
Immunotherapy represents a major paradigm shift in cancer therapeutics. Anti-cytotoxic T-lymphocyte antigen-4 (anti-CTLA-4), and anti-programmed death-1 receptor (anti-PD-1) are checkpoint inhibitors that block tumor pathways that inactivate cytotoxic T cells (**Figure 4**). Restoring the ability of the innate and adaptive immune system to eliminate tumor cells may lead to more durable clinical responses in a variety of tumor types, including melanoma, non-small cell lung cancer, bladder cancer, renal cancer, and Hodgkin lymphoma.<sup>26-31</sup> In patients with metastatic melanoma, overall response rates range from 12% with CTLA-4 inhibitors to 40% with anti-PD-1 agents.<sup>32</sup> Given the cost of immunotherapy, biomarkers that can identify patients who will likely respond to these treatments are needed. Recently, 2 powerful biomarkers were identified using NGS analysis: tumor mutational burden (TMB), and microsatellite instability (MSI).



**Figure 4.** Checkpoint-inhibiting antibodies block the interactions of CTLA-4 and PD-1 with their ligands, resulting in reactivation of cytotoxic T cells. Reprinted by permission from Macmillan Publishers Ltd: Drake CG, et al. *Nat Rev Clin Oncol*. 2014;11(1):24-37, copyright 2014.<sup>33</sup>

## NGS analysis for tumor mutational burden (TMB)

TMB measures the number of nonsynonymous (protein changing) mutations identified per megabase of DNA. A recent study used WES data collected from approximately 6000 cases showed a wide range of TMB across many cancers, with lung cancer and melanoma having the highest amounts, consistent with environmental exposures that contribute to tumorigenesis, such as smoking and ultraviolet light (**Figure 5**).<sup>34,35</sup> TMB has been analyzed as a predictive biomarker for response to checkpoint inhibitor immunotherapy. Cancers with high TMB have been shown to be associated with improved objective response rates (ORRs), durable clinical benefit, and improved progression-free survival (PFS).<sup>36-38</sup>



**Figure 5.** Graph showing the prevalence of mutations across many cancer types. Each tumor sample (dots) was compared with the median mutation frequency (red horizontal lines). Cancer types are listed in order from the lowest TMB prevalence (vertical axis, log scale) to the highest prevalence on the right. ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia. Reprinted by permission from Macmillan Publishers Ltd: Alexandrov LB, et al. *Nature*. 2013;500(7463):415-421, copyright 2013.<sup>34</sup>

## NGS for detection of microsatellite instability

Microsatellites are short, 2– to 5–base pair DNA sequences that are tandemly repeated from 10 to 60 times.<sup>39</sup> These regions are prone to base-pair mismatching during DNA replication but can be corrected by DNA mismatch repair (MMR) proteins. If MMR is impaired, genetic hypermutability and MSI can result. For example, MSI has been observed in approximately 15% to 20% of colorectal cancers (CRCs).<sup>40</sup> One study compared treatment with the PD-1 inhibitor pembrolizumab for CRCs with high levels of MSI (MSI-H) vs those without MSI. MSI-H tumors showed an ORR of 40% and disease control rate (DCR) of 90%. In contrast, patients with MSI stable tumors had an ORR of 0% and DCR of 11%.<sup>41</sup> Several studies have shown that NGS can enable highly accurate detection of MSI and may have advantages over traditional PCR-based methods, including the ability to test large batches of samples and provide a more standardized interpretation and additional important information beyond that provided by PCR.<sup>42,43</sup> MSI has significant implications for tumor etiology, prognosis, therapeutic choices, and familial cancer risk (eg, Lynch syndrome).<sup>40</sup>

## Summary

Over the last decade, advances in medical genomics have elevated the understanding of cancer biology and led to novel approaches for the diagnosis, management, and treatment of patients with cancer. The number of healthcare practitioners adopting NGS continues to grow, as does the recognition of the power of NGS to offer improved sensitivity and specificity, greater genetic coverage, and the ability to use small quantities of tissue. By enabling the simultaneous detection of multiple types of genetic alterations and the ability to pool samples during one sequencing run, TAT for results in laboratories using NGS has decreased. In contrast to traditional molecular techniques, NGS has the potential for more specific, individualized, patient assessment. As results from multiple clinical trials have shown, the integration of genomic information into patient treatment plans can lead to shortened time to diagnosis, more targeted therapeutic strategies, and ultimately, improved outcomes for patients.



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# Next-Generation Sequencing (NGS): The Future of Genomic Medicine Is Here

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