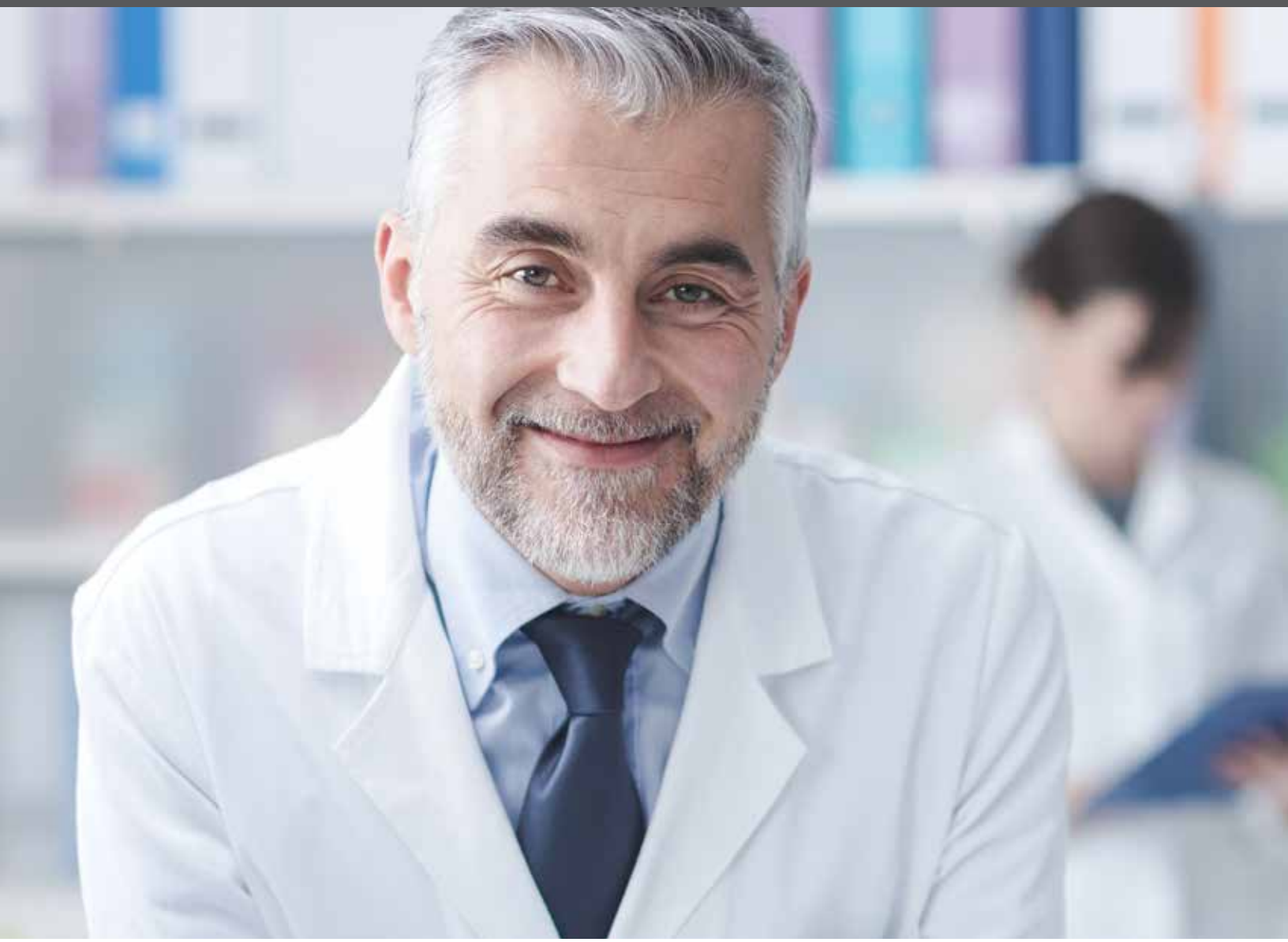
ion torrent



An introduction to next-generation sequencing for precision oncology

ThermoFisher SCIENTIFIC

Disease of the genome

It is widely recognized that cancer is a disease of the genome, resulting from genetic alterations in several classes of genes that break down critical cellular processes governing cell growth and differentiation. Among these genes are tumor suppressor genes, which function to protect cells from cancer, and oncogenes, which govern cell differentiation and replication but can also drive cancer progression when altered by mutations. As these gene mutations accumulate, they simultaneously inactivate cell protection processes and over-activate cell replication and differentiation. At the heart of precision oncology is the need to understand these so-called driver mutations to gain insights into potential targets for therapeutic intervention.

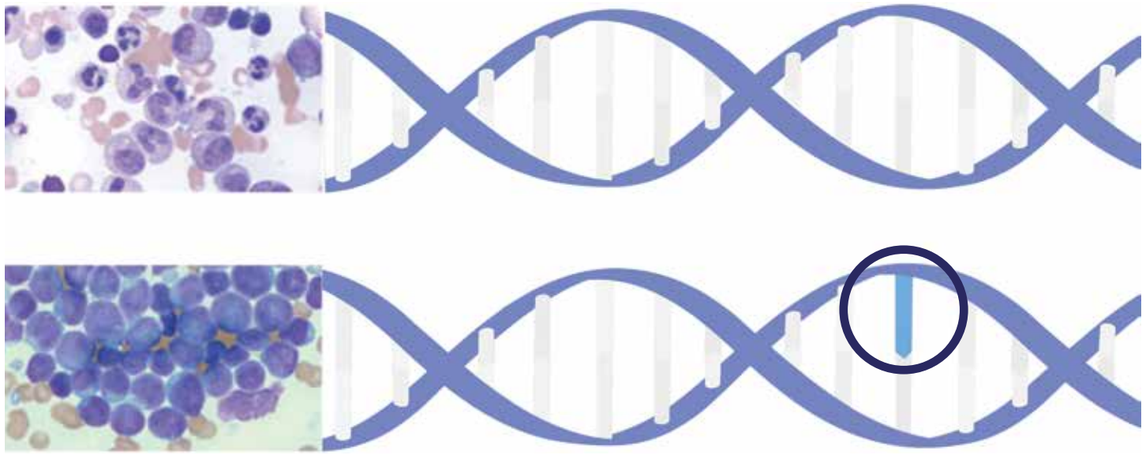


Figure 1. Example of a genetic alteration (single-base pair change) that enables cells to bypass the processes that govern cell growth and differentiation, resulting in unrestricted cell division.

Precision oncology—it's personal and targeted

Precision oncology is the new paradigm of cancer care. In this field, the cancer biomarkers in an individual patient are identified through molecular profiling. Once these biomarkers are identified they are matched with therapeutics that specifically target associated biological pathways, resulting in uniquely tailored, personalized care plans that hold the promise of optimizing outcomes.

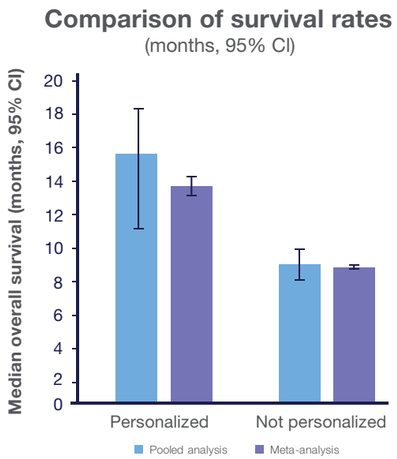


Figure 2. Precision oncology optimizes outcomes. Source: Schwaederle M, Zhao M, Lee JJ et al. (2015) Impact of Precision Medicine in Diverse Cancers: A Meta-Analysis of Phase II Clinical Trials. *J Clin Oncol* 33:32:3817-3825.

Available targeted therapies

Over the last 20 years, the development of targeted therapies has accelerated, and a large number of them are currently available. Testing for relevant, actionable genetic alterations (biomarkers) has become a necessary and routine part of the oncology patient management process.

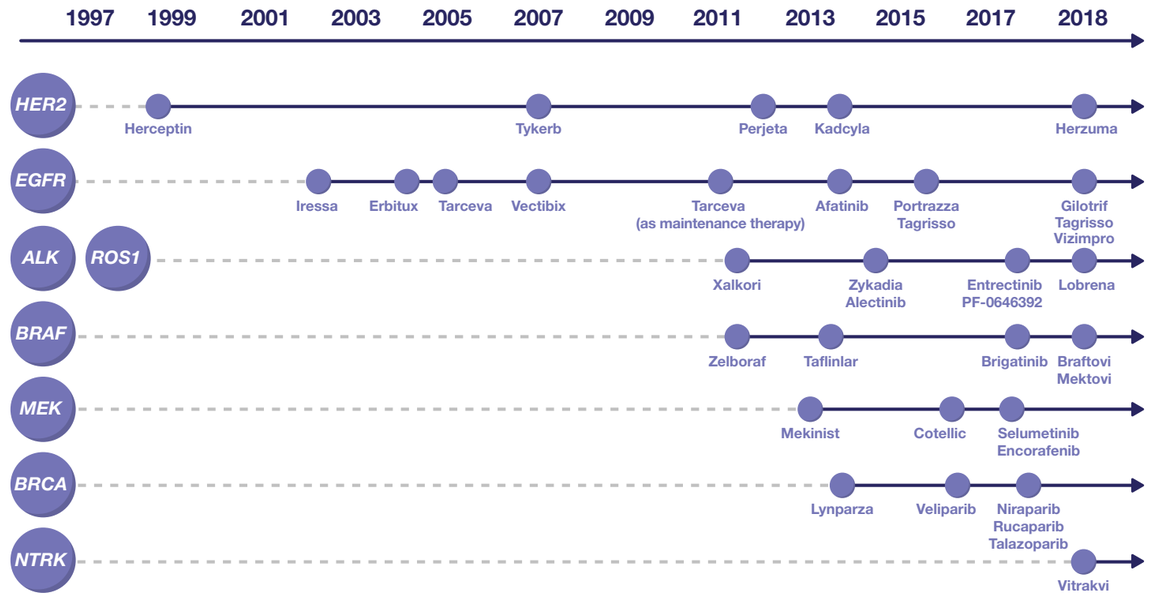


Figure 3. Compiled from FDA website on approvals for targeted therapies. Does not include all combination approvals.

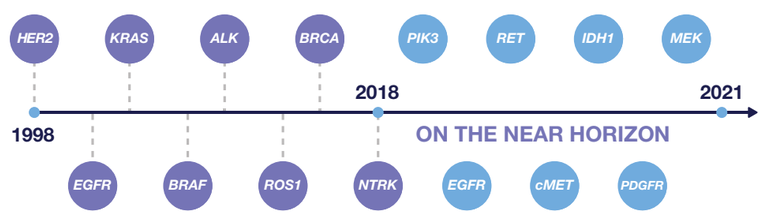
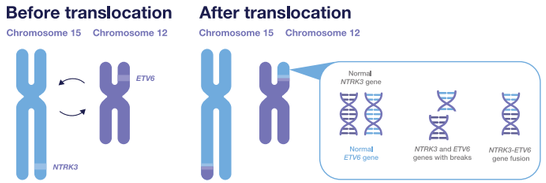


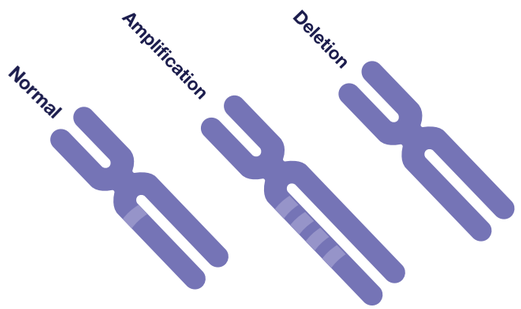
Figure 4. While it took 20 years for first 10 biomarkers to be established, development is currently accelerating.

Over 73% of oncology treatments in the pharmaceutical pipeline are precision therapies. And consequently, there's a growing demand for high-quality, efficient, and effective biomarker testing, and for pathologists to play a more active and integral role on the patient's care team.

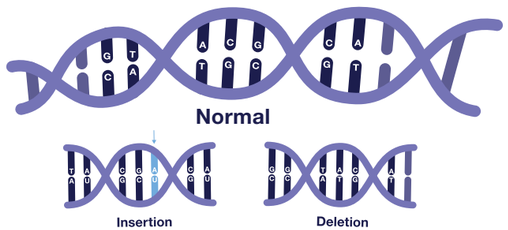
Different types of genomic aberrations can be detected by NGS simultaneously from one sample



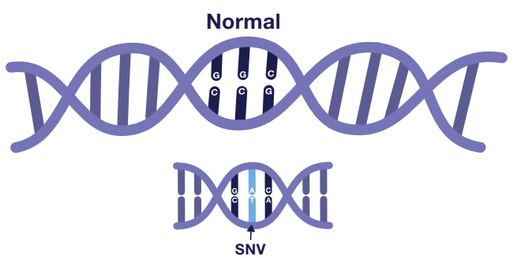
1. **Translocations** (migration of the DNA from one chromosome to another) leading to the origination of new fusion genes and proteins play a significant role in early cancer development. Yet, only those that get transcribed to RNA can potentially become functional, and therefore RNA sequencing is the preferred method for fusion testing.



2. **Constitutional copy number variants (CNVs)** are DNA segments present at a variable copy number in comparison to a normal genome. Amplification of oncogenes and deletion of tumor suppressor genes are often observed in tumor cells as drivers of oncogenesis, allowing for increased production of specific proteins for cancer formation and growth. Many examples have been identified in various cancers: amplification of *EGFR* in gliomas, *MYCN* in neuroblastomas, *MYC* in myeloid leukemia, and *ERBB2* in breast, ovarian, and lung cancers are often associated with unfavorable prognosis and drug resistance.



3. **Insertions and deletions (indels)** are additions or deletions of one or more nucleotides in a DNA sequence. They are the second most common aberrations in the human genome (after SNPs) and are well known to contribute to cancer development (e.g., a group of deletions and insertions in the *EGFR* gene in NSCLC). Activating *EGFR* mutations are inherently sensitive to *EGFR* inhibitors.



4. **Single nucleotide variants (SNVs)** are single-base changes in DNA sequences responsible for genetic diversity but also possibly contribute to development of complex diseases such as cancer. They are most frequent in certain tumor types. For example, the BRAF V600E mutation in melanoma is a substitution at the second position of codon 600 of exon 15 (GTG>GAG), c.1799T>A, that results in an amino acid change from valine (V) to glutamic acid (E). These variants lead to over-activation of the *MAPK* pathway, one of the mechanisms controlling basic cellular processes such as growth, proliferation, and apoptosis.

The vital role of NGS in precision oncology

Obtaining a complete genetic profile with single-gene testing is problematic and impractical for several reasons—time, tissue, and costs. With a single-gene approach, it can take several weeks to get the test results for all genes of interest, which leads to treatment delays, a suboptimal scenario for any cancer patient, but especially for those with late-stage disease. In addition, each single-gene test depletes the patient's precious tissue samples, often necessitating additional biopsies and compounding the burden on the patient's quality of life and health. By providing comprehensive insight into genetic biomarkers, next-generation sequencing (NGS) is accelerating advancements in precision oncology. NGS offers several advantages over legacy diagnostic methods that typically test for single biomarkers, sequentially.

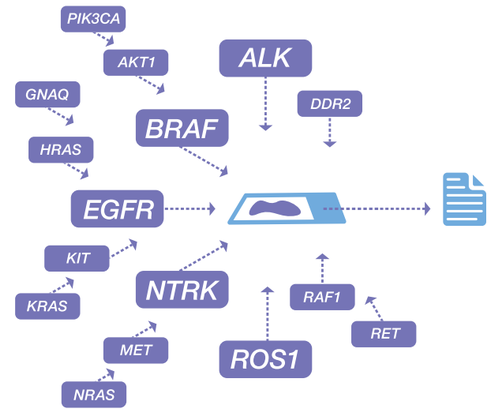


Figure 5. Using NGS, providers can analyze many biomarkers simultaneously with one test and one report.

Key advantages of NGS for precision oncology applications

NGS can analyze many biomarkers and biomarker types (for example, mutations, fusions, copy number variations) simultaneously with a single test.

Time savings: A comprehensive molecular profile can be available in just days, empowering clinicians with critical biomarker data so individualized treatment decisions can be made sooner.[11]

Tissue savings: NGS can provide comprehensive genomic profile results from one small sample[5-10] (as little as 10 ng of DNA or RNA).[12]

High specificity and sensitivity enables detection of genomic variants even if they are present in extremely low fractions of cells in the sample (such as in liquid biopsies), and allows clinicians to distinguish between possible different clones of tumor cells.[12]

Cost and resource savings: If three or more biomarkers are required, it becomes cheaper to run one NGS test than multiple single-gene methods. NGS also allows savings by streamlining training and technical experience requirements, as well as instrumentation maintenance, on one platform, which also saves on space requirements.[13, 22]

Reduced sample handling errors: The more tests that are performed to achieve the required result, the more potential for errors to occur. Consolidation into one NGS test instead can reduce those rates.[17, 18]

Importance of the high specificity and sensitivity of NGS

Among the key advantages of NGS are the high sensitivity and specificity of the assays. High specificity is important because cancers are often a heterogeneous disease. It is acknowledged that while progressing, subclonal populations of cancer cells can arise, carrying different mutation signatures. In a given tumor sample, therecould be a mixed population of these subclones, which need to be evaluated individually. The high sensitivity of NGS can resolve the proportion of different alleles, or variations of particular DNA bases, present in the sample—even when a given population of cells is present at a very low frequency. This information can help the care teams understand how the disease is evolving and ultimately inform decisions about the optimalcourse of therapy.

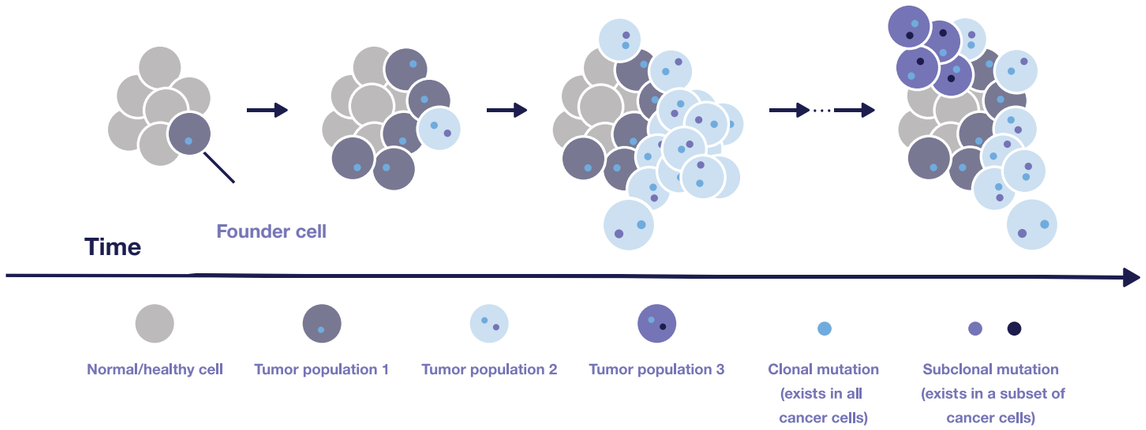


Figure 6. Heterogeneous cancer cell populations. As cancer progresses, subpopulations of cells can develop that harbor additional mutations. NGS can detect variants in heterogeneous populations of cells, including those that exist at very low frequencies.

The tissue is still an issue

Low input requirements of some NGS methods, coupled with the ability to use a single test for multiple biomarkers (Figure 5), translate to a drastic reduction in the amount of tissue required for testing. This is good news, since the tissue samples themselves are often very small (Figure 7). However, not all NGS methods have the same low sample requirements, and it is important to check when deciding which to implement.

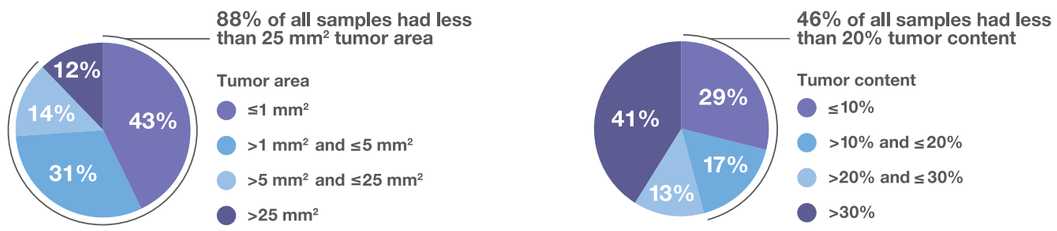
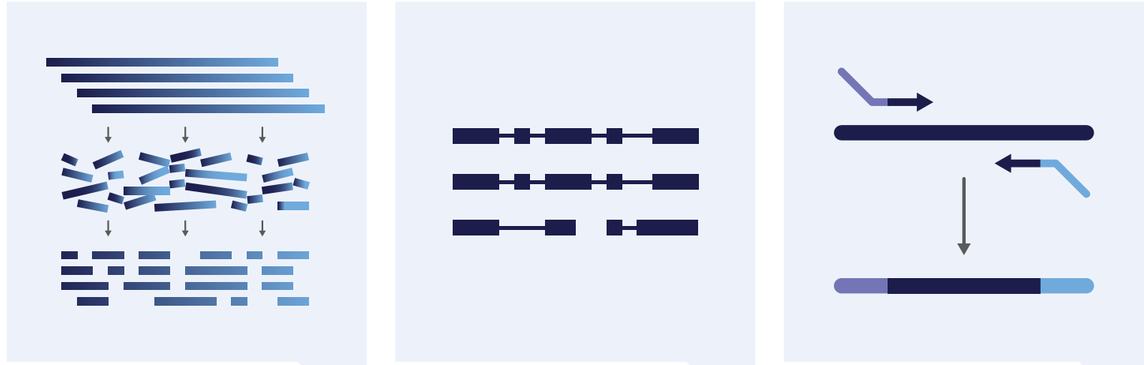


Figure 7. Many routine clinical NSCLC samples do not have enough material for traditional single-gene testing methods. Analysis of 1791 NSCLC samples received by Cancer Genetic Inc. during one year.[19]

Next-generation sequencing methods

NGS is a highly scalable technology—the spectrum of analysis can extend from a small number of genes to an entire genome, depending on the goal. For instance, as their name implies, the massive scale of whole-genome sequencing (WGS), whole-exome sequencing (WES), and whole-transcriptome sequencing makes them the most appropriate methods for research and discovery endeavors. Targeted applications, such as solid tumor profiling and liquid biopsy (circulating tumor cells and cell-free DNA), are better suited for the clinical arena.



Whole-genome and whole-exome sequencing

Whole-genome sequencing (WGS) and whole-exome sequencing (WES) provide the identity of every DNA base across the genome and exome, respectively. The primary drawbacks of these methods from a clinical perspective are the sheer quantity of data and the challenge of interpreting it. The costs associated with the analysis and storage of these massive data sets are much higher, and ultimately, the data is not very actionable. Additionally, there are ethical concerns associated with the handling of incidental findings of unknown clinical significance that can occur with WGS and WES.

Whole-transcriptome sequencing

Whole-transcriptome sequencing provides sequence information about coding and multiple noncoding forms of RNA to assess variations and gene expression levels across the entire transcriptome. While whole-transcriptome sequencing can offer valuable insights for cancer analysis including detection of novel gene fusions, given its large scale it has the same drawbacks as WGS and WES—and therefore is better suited for research and discovery.

Targeted sequencing

Targeted sequencing covers a relatively small set of genes or targeted regions of interest. Typically, an assay panel will feature genes with known clinical relevance. The advantage of using these targeted panels is that they can be designed for specific specimen and cancer types, such as solid tumors or hematologic malignancies. The panels can contain coverage for hotspot mutations, which are mutations with high recurrence for a particular tumor type, or they can cover whole gene-coding regions. Targeted assays can be designed for both DNA and RNA analysis. The fast turnaround time, low cost, low sample input requirement, and relative ease of interpretation make targeted sequencing particularly well suited for clinical applications.

Clinical applications of targeted sequencing in oncology

Solid tumor profiling from FFPE tissue

The most common clinical application of NGS is solid tumor profiling from tissue specimens derived from a tumor resection, biopsy, or cytological specimens such as fine needle aspirate (FNA) that have been formalin-fixed and paraffin-embedded (FFPE). Despite the latest advances in using liquid biopsy, FFPE tissue testing remains the gold standard approach for initial profiling of the tumor, if tissue is available. Yet, it is important to understand that the nucleic acids degrade during the fixation and paraffin embedding process, so a correctly executed pre-analytical phase is key to obtaining good results from FFPE tissue. NGS methods that require little of the sample while still robustly performing with FFPE tissue are preferable.

Tumor mutational burden and other emerging immuno-oncology biomarkers

Tumor mutational burden (TMB) is an emerging biomarker that is rapidly gaining traction in the immuno-oncology field. TMB is a measurement of the number of mutations harbored in tumor cells. Clinical studies demonstrate that patients with a higher TMB have a higher probability of responding to certain immunotherapies. While not yet in routine clinical use, TMB is likely to become one of the standard biomarkers to be tested in the near future.

Learn more about TMB at oncomine.com/academy

Solid tumor profiling from liquid biopsy CTCs or cfDNA

Another method of assessing cancer biomarkers with NGS uses a simple blood sample—a liquid biopsy. The blood of a patient with cancer carries circulating tumor cells (CTCs) and cell-free DNA (cfDNA), which originate from the solid tumor or its metastatic lesions. In cases when the original FFPE block is not available and it is impossible to obtain a new biopsy sample, liquid biopsy can serve as an alternative material for initial profiling. Also, minimally invasive liquid biopsies can be taken at regular intervals to monitor therapy response and resistance development. There is evidence that liquid biopsies can help detect cancer cells earlier at metathesis, the colonization phase that leads to metastatic tumors[2].

Hematological tumor profiling

Assay panels can also be designed to profile hematological malignancies, including major myeloid disorders: acute myeloid leukemia (AML), myeloid dysplastic syndrome (MDS), myeloproliferative neoplasms (MPN), chronic myeloid leukemia (CML), chronic myelomonocytic leukemia (CMML), and juvenile myelomonocytic leukemia (JMML). As with solid tumor profiling, the genes of interest are selected based on clinical relevance; but in this case, the nucleic acid is extracted from a blood sample instead of tumor tissue.

Infantile fibrosarcoma	91%–100%	**Thyroid cancer**	2%–12%	**Astocytoma**	3%
Secretory breast carcinoma	92%	**High-grade gliomas (pediatric)**	10%	**Lung cancer**	0.2%–3%
Spitzoid melanoma	16%	**Melanoma, high-grade glioma, renal cell carcinoma, sarcoma; pancreatic and breast cancer**	<5%	**Colon cancer**	1%
		Cholangiocarcinoma	4%	**Sarcoma**	1%
				Glioblastoma	1%
				Head and neck squamous cell carcinoma	0.5%

Figure 8. Estimated frequency of *NTRK* gene fusions in specific tumor types.

How targeted NGS works

Selecting assay targets
The first step with targeted sequencing is selecting the gene content for your assay panel. Assay content can range from single point mutations to full genes or other regions of interest. For example, a lung cancer panel would likely cover disease-associated genes like *EGRF*, *ALK*, *ROS1*, and *BRAF*, as well as emerging biomarkers currently in clinical trials.

Fortunately for the novice user, there are a number of predesigned assay panels available that feature rigorously selected, potentially clinically relevant content for specific disease types. These fixed panels and software tools make it easy for any laboratory to run an NGS test and generate a report that identifies relevant biomarkers, associated therapies, related clinical trials, and guidelines as applicable.

Amplicon-based next-generation sequencing principle

1. DNA and RNA is extracted from FFPE sample
2. Regions of interest are amplified from fragmented DNA and barcode labeled
3. Each labeled fragment is attached to its own bead and copied, until it covers the bead
4. Prepared DNA fragments, called amplicons, are sequenced in a massively parallel fashion
5. Hydrogen ions are released, changing the solution pH, as complementary nucleotides are added
6. pH changes are converted to voltage changed and measured with a volt-meter
7. Integrated software assembles and analyzes the data to identify target gene variants, if present in the sequence

New trend: “organ agnostic” biomarker testing

While most of the currently used biomarker variants are typical for one tumor type, some genomic variations have been proven to cause cancer in multiple organs, which is changing the paradigm for how the disease is tested and treated. These types of variations illustrate how cancer is truly a disease of the genome, not an organ disease. In *NTRK* gene fusions, for example, the TRK family of proteins (oncogenic drivers) are fused with an unrelated gene that causes permanent activation and overexpression. *NTRK* fusions are associated with a broad range of tumor types.

The NGS workflow—streamlined for ease and efficiency

Prepare sample and library
Begin the NGS workflow by preparing a sequencing library from the tissue sample. First, extract and purify the DNA and/or RNA, depending on the assay.

Using PCR, amplify regions of interest to generate an amplicon sequencing library. You can either prepare a library from a single specimen, or combine multiple specimens into a mixed library. In a process called sample multiplexing, each specimen is tagged with a specific barcode so they can be analyzed independently downstream. Multiplexing enables you to optimize efficiency by maximizing the number of samples processed in each sequencing run.

Create template
After the sequencing library is prepared, generate a template in preparation for sequencing. This entire process can be easily automated. During template generation, the library is settled onto a solid substrate and further amplified. With Ion Torrent™ technology, the substrate is a semi-conductor microchip that enables the sequence of each amplicon in the library to be read independently.

Sequence
Once the template is ready, simply load the chip into the sequencer and initiate a sequencing run. The sequence of each amplicon in the library will be read during this process and the data will be digitally transmitted to a computer for downstream analysis.

Analyze data and interpret results
Integrated analysis software assembles the amplicon sequencing data and calls any identified variants. Using a decision support tool, gene variants detected in the sequences can be matched against databases of known relevant biomarkers, associated therapies, clinical trials, and guidelines.

See a sample report

How to select the optimal NGS method

While NGS is the ideal platform for testing multiple biomarkers and preserving precious sample tissue, not all NGS is the same. These are the five key factors to consider when choosing the NGS technology for your laboratory:

- **Adequate portfolio of applications and panel coverage**—In precision oncology, one size does not fit all. For example, if you are routinely testing NSCLC samples, you need a panel that includes *ALK, ROS1, EGFE, BRAF, NTRK, RET* and likely also offers the possibility to profile liquid biopsy samples, as in many cases there is not enough tissue available. For immuno-oncology clinical research, not only TMB but also T-cell receptor (TCR) sequencing is becoming important. In general, precision oncology testing as a discipline is developing fast, so a supplier who is offering an adequate breadth of portfolio and constant innovation is preferable.
- **Panel design**—Depending on the variant to be detected, either DNA- or RNA- based sequencing is preferable. Often, an assay that can do both at once is required. RNA-based methodology is optimal for testing fusions such as *NTRK,* as it directly detects translocation events between the *NTRK* gene and partner gene. It should also be able to detect not only all the known, but also novel driver and partner combinations.
- **Sample requirements**—Tissue is still the issue, and often the amount available is very limited. Different NGS methods vary significantly in the amount they require, ranging from 10–500 ng of nucleic acid (RNA or DNA), which can have a direct impact on your ability to successfully test all samples.
- **Completeness and automation level of the workflow**—NGS workflows can be complex. An easy-to-use and highly automated workflow from sample to report simplifies lab operation and test implementation.
- **Analytical validation support**—High-touch consultation service and support from the vendor helps accelerate a lab's validation process to implement the test in a time-efficient manner and save costs.
- **Robustness and key performance characteristics**—Not all NGS technologies can handle all tissue types equally well. For example, FFPE material can be challenging, especially when not enough tumor sample is present. Look for evidence of the sequencing success rate, or the opposite–failure rate and Quantity Not Sufficient frequency. As discussed in this guide, specificity and sensitivity are very important, as the tumor cell population may be heterogeneous, and the particular aberration may be present only in small proportion, while the patient might still benefit from the corresponding therapy. High reproducibility is also required, so you can generate consistent results.

ion torrent

Conclusion

The shift toward precision oncology is rapidly gaining momentum, fueled by a growing knowledge base of cancer genomics and biology. With genomic profiling now becoming the standard of care, NGS has emerged as the primary platform for cancer biomarker detection. Many countries already have regulatory-approved NGS tests with reimbursement coverage available from insurance payers or universal health care systems. The new paradigm of precision oncology holds the promise of a future where patients only receive therapeutics that align to their individual cancer, and where they experience enhanced care and achieve more successful outcomes [10].

Ready to help fulfill the promise of precision oncology? Start the NGS conversation with your colleagues today and reach out to our representatives at **oncomine.com** for advice.

References

1. Surrey L, Luo M, Chang F, et al. (2016) The Genomic Era of Clinical Oncology: Integrated Genomic Analysis for Precision Cancer Care. *Cytogenet and Genome Res* 150:162-175.
2. Kamps R, Brandao R, ven den Bosch B, et al. (2017) Next-Generation Sequencing in Oncology: Genetic Diagnosis, Risk Prediction, and Cancer Classificaton. *Int J Mol Sci* 18:308.
3. Personalised Medicine Coalition. THE PERSONALISED MEDICINE REPORT. 2017.
4. CAP TODAY. NGS to take top spot as cancer biomarker testing broadens. 2018.
5. Zhang J, Späth S, Marjani S, et al. (2018) Characterization of cancer genomic heterogeneity by next-generation sequencing advances precision medicine in cancer treatment. *Precis Clin Med* 1(1):29-48.
6. Meldrum C, Doyle M, Tothill R (2011) Next-Generation Sequencing for Cancer Diagnostics: a Practical Perspective. *Clin Biochem Rev* 32:177-195.
7. Heitzer E, Perakis S, Geigl J, et al. (2017) The potential of liquid biopsies for early detection of cancer. *NPJ Precis Oncol* 1:36.
8. Goodman A, Kato S, Bazhenova L, et al. (2017) Tumor Mutational Burden as an Independent Predictor of Response to Immunotherapy in Diverse Cancers. *Mol Cancer Ther* 16(11):2598-2608.
9. Vaishnavi A, Le AT, Doebele RC (2015) TRKing down an old oncogene in a new era of targeted therapy. *Cancer Discov* 5(1):25-34.
10. Gagan J, Van Allen E. (2016) Next-generation Sequencing to Guide Cancer Therapy. *Genome Med* 7:80.
11. Miller TE et al. (2018) Clinical utility of reflex testing using focused next generation sequencing for management of patients with advanced lung adenocarcinoma. *J Clin Pathol.* 0:1-7.
12. Moore D. Tissue is still the issue. *The Pathologist.*
13. Clinical applicability and cost of a 46-gene panel for genomic analysis of solid tumours: Retrospective validation and prospective audit in the UK National Health Service. *PLOS Medicine.* February 14, 2017.
14. NSCLC example: NCCN Guidelines v13,2017. Metastatic Disease.
15. Tiany MY et al. (2018) Multiple Biomarker Testing Tissue Consumption and Completion Rates With Single-gene Tests and Investigational Use of Oncomine Dx Target Test for Advanced Non Small-cell Lung Cancer: A Single-center Analysis. Clinical Lung Cancer. 1-10.
16. Paolini D et al. (2018) Ventana ALK (D5F3) in the Detection of Patients Affected by Anaplastic Lymphoma Kinase-positive Non–Small-cell Lung Cancer: Clinical and Budget Effect. *Clinical Lung Cancer.* 19:e735–e743.
17. Patton S et al. (2014) Assessing standardization of molecular testing for non-small-cell lung cancer: results of a worldwide external quality assessment (EQA) scheme for EGFR mutation testing. *Br J Cancer.* 111:413-20.
18. Kapp JR et al. (2015) Variation in pre-PCR processing of FFPE samples leads to discrepancies in BRAF and EGFR mutation detection: a diagnostic RING trial. J Clin Pathol. 68:111-8.
19. Audit of 1791 NSCLC samples in Cancer Genetic Inc. laboratory. NGS to take top spot as cancer biomarker testing broadens. *CAP TODAY*, June 2018.
20. Sciavolina P. (2015) Tropomyosin-Related Kinases (TRK) Making Headway in Head and Neck Cancers. *Targeted Oncology.*
21. Cocco E. (2018) NTRK fusion-positive cancers and TRK inhibitor therapy. *Nature Reviews Clinical Oncology.*
22. Miller TE, et al. (2018). Clinical utility of reflex testing using focused next-generation sequencing for management of patients with advanced lung adenocarcinoma. *J Clin Pathol.* 0:1–7.

Learn more at **oncomine.com**

ThermoFisher SCIENTIFIC