What exactly does the term next-generation sequencing refer to?

First, I will offer a note regarding this term. Although next-generation sequencing has become a ubiquitous catchphrase, what we are referring to is DNA sequencing that occurs in what is known as a massively parallel format, which is the term often used in the scientific literature. The terms are equivalent, however.

Both next-generation sequencing and massively parallel sequencing refer to a novel DNA sequencing process that has been made possible by hardware and software engineering advances in the past decade. In the past, we would sequence and analyze 1 gene at a time. With massively parallel technologies, tens of thousands of genes can be sequenced simultaneously. We also now have software that can read these tens of thousands of DNA sequence strands simultaneously. First we obtain sequences containing millions of base pairs, and then we use sophisticated bioinformatics algorithms to determine exactly where a stretch of sequence (a read) aligns (or maps) to the human genome (or fraction of the genome). The basic sequencing chemistry is not much different than what it has been for decades—enzyme-mediated incorporation of sequential nucleotides—but the scale of that sequence, enhanced by novel hardware and software technology, is now much more advanced.

What is the difference between targeted sequencing approaches and whole-genome or whole-exome approaches?

With targeted sequencing, a laboratory designs a sequencing panel of a limited number of target genes, usually ranging between 10 and 500, and only the genes within this panel are sequenced. This targeted approach is most commonly undertaken when a laboratory or clinician only wants to investigate actionable mutations in a certain tumor type, best defined as mutations that have been previously shown to have direct diagnostic, therapeutic, or prognostic relevance. A mutation that is directly targeted by a gene-specific inhibitor drug (a tyrosine kinase inhibitor in non-small cell lung cancer with an epidermal growth factor receptor [EGFR] mutation, for example) would be an ideal example of an actionable mutation. EGFR mutations are strong predictors of efficacy for the EGFR tyrosine kinase inhibitors, including the first-generation drugs erlotinib (Tarceva, Genentech/Astellas) and gefitinib (Iressa, AstraZeneca; limited distribution) and the second-generation drug afatinib (Gilotrif, Boehringer Ingelheim).

At the moment, in the clinical diagnostic arena, the targeted sequencing approach is much more common than the alternative “wide-net” sequencing approach, which would include whole-genome or whole-exome sequencing. With whole-exome sequencing, for example, all of the approximately 20,000 protein-coding genes are examined, not just the 10 to 500 genes likely to be actionable in the specific tumor at hand.

Obviously, these nontargeted, wide-net sequencing approaches generate an enormous amount of data, require more resources upfront, and are much more costly compared with targeted sequencing. In addition, the sheer quantity of data can make it very difficult and time-consuming to figure out how to convert the vast raw sequence into something clinically relevant to the patient. But there are important advantages, too. Most importantly, particularly in the discovery (not clinical...
diagnostic) arena, casting a wider net in the search for genes involved in a particular type of cancer may lead to important pathogenic, diagnostic, or therapeutic advances. With whole-exome sequencing, there is no bias in considering what genes may or may not be involved.

H&O Are there cases in which whole-exome sequencing is the best approach for finding genetic mutations relevant to various types of cancer?

RP For early research, yes, whole-exome sequencing is likely better because it allows one to discover genetic variants that were not previously known. In my view, however, targeted sequencing is better for routine clinical care and practical decision-making in oncology. The targeted panels used for sequencing tumor samples from routine oncology patients, by definition, will include only genes that are known to have actionable clinical consequences—and with a reimbursable cost and fast turnaround time. Most of the academic medical centers and commercial laboratories offering this service in clinical oncology are taking the targeted approach.

H&O Cancer appears to be leading the way in moving sequencing forward in both the research laboratory and the clinic. Why is that?

RP During the past couple of decades, it has become increasingly apparent that cancer is a disease with sequential insults to the genome as the main cause. Next-generation sequencing is enabling us to find and catalog all of these insults to the genome. That is the simple part. The hard part is figuring out what all these cataloged variants mean for diagnosis, prognosis, and treatment.

Today, we can take a sample from a tumor and fairly quickly identify the specific changes present in the cancer genome. But how many of those changes are clinically relevant? How many are of no practical consequence? How many can be targeted with a drug? How many have prognostic relevance?

Another purely practical reason why cancer is often at the forefront in this field is that researchers and clinicians have been so careful about saving tumor samples for potential future studies. So, for example, if I want to study a particular type of cancer, I do not have to wait for the next 50 patients with that type of cancer to come into the clinic. Instead, after institutional review board approval, I can walk down the hall to where we have thousands of tumor samples stored and well characterized. Archived tumor biorepository samples means there is already DNA sitting on the shelf, which is greatly expediting research.

H&O Concluding that a particular mutation is actionable and therefore possibly clinically relevant seems to be a somewhat controversial area of cancer research. What are the issues at play?

RP On the one hand, there are oncologists who want to have their patient’s tumors comprehensively characterized. As a broad generalization, these oncologists are hoping that this sequencing information will be directly clinically beneficial, in real time, for the patient being studied. However, even if not of immediate practical relevance, this genomic information may be useful in the near- or long-term future—if not for this patient, perhaps for others to follow.

On the other hand, there are oncologists who are not very interested in tumor sequencing. We can indeed describe the mutations present in a set of genes within the tumor, but if there are no US Food and Drug Administration (FDA)-approved drugs available to treat those mutations, then what is the point?

Both sides are valid, of course, but in 2014, the reality is that there are not that many rigorously defined actionable mutations that are linked to FDA-approved oncology drugs. There are, however, numerous pipeline mutations, genetic variants that have been identified and are in the early stages of becoming targetable. The presence of such a pipeline mutation, for example, could allow a patient to enroll in a clinical trial of a gene-targeted drug. However, even these early-stage findings are currently the exception rather than the rule. The larger category of actionable mutations refers to those that are known to contribute to tumor pathogenesis, and may allow more accurate tumor diagnosis, classification, or prognosis, but for whom no targetable pharmaceutical agent is (yet) available.

The obvious exception here is the BCR/ABL fusion gene. This mutation is present in nearly all cases of chronic myeloid leukemia, and is primarily responsible for triggering the disease. This discovery, which long predates next-generation sequencing, is still the paradigm for personalized cancer medicine: discover the genetic alteration that is driving the cancer, and then create a drug to target that mutation.

It is very difficult to discern between driver mutations and passenger mutations; that is, the mutations responsible for the initiation and progression of the cancer cell vs those that may be present in a tumor but are not contributing to its growth. The fact that mutations arise and evolve as cancer progresses further complicates matters. A tumor sequenced 2 years after it was first discovered may contain mutations that were not present at diagnosis.
**H&O** What are some more recent examples of clinically applicable data derived from next-generation sequencing?

**RP** Perhaps the best example is acute myeloid leukemia (AML), which was the first cancer to have been completely characterized at the genomic sequence level. There are now several AML prognostic genes that are very well documented, and some of which are direct drug targets. For example, AML patients with a particular variant of the FLT3 gene tend to have a higher rate of relapse after initial therapy compared with AML patients without this mutation, thus perhaps justifying more aggressive treatments. Other clinically relevant cancer genomic findings include mutations in the EGFR receptor in lung cancer, HER2/neu in breast cancer, c-KIT in gastrointestinal stromal tumors, and BRAF in melanoma.

Many of the genetic mutations identified through next-generation sequencing are actively being investigated in clinical trials and are not yet part of routine treatment. However, although targeting a mutation with a drug is the obvious primary goal, the relevance of a genetic variant to a patient’s diagnosis and prognosis is also very important.

**H&O** How useful is the prognostic information in clinical care?

**RP** As with the popularity of tumor sequencing, this area also seems to be highly variable. Many oncologists do not factor in the value of this prognostic information because they believe it may not directly impact patient care. But a genetic mutation with prognostic relevance may, in fact, be very valuable in clinical care. If, for example, a patient has a mutation associated with a survival time of 10 months vs 10 years, then that may impact treatment decisions, even if the mutation itself is not targetable. In other words, the risk to benefit balance of a particular therapy can be tailored based on prognostic genetic markers—with riskier treatments more easily justified in a tumor predicted to be aggressive based on its genomic profile.

AML provides an illustrative example. If a patient has a genetic profile classified as high-risk AML, then recommending a stem cell transplant upon remission is much more justifiable. Transplants are risky procedures, so they would not necessarily be recommended for patients with low-risk AML, for which other treatment options may be preferable to the 10% risk of death associated with transplants. But for high-risk patients, that risk is worthwhile because if the procedure is successful, the patient may be cured.

So right now the clinical implementation of next-generation sequencing is not just limited to selecting a drug to target a given mutation. Rather, the information is being used in a more nuanced way in treatment decisions.

**H&O** Is cost a barrier to more widespread clinical implementation of next-generation sequencing?

**RP** Yes, cost and timeliness are both barriers. Sequencing a panel of targeted genes costs approximately $2000 to $4000. Obtaining these complex results usually takes about 2 to 4 weeks, which can be a long time in a clinical setting. Labs that perform sequencing are also having a hard time getting reimbursed for this service. Insurers are not paying for sequencing of many genes, and even those that are covered are often paid at a level below the cost of generating the sequence. Many laboratories thus have no fiscal incentive to offer next-generation sequencing tests, and thus the availability of this service may soon become restricted.

**H&O** Is this hurdle slowing down research?

**RP** No, the lack of insurance coverage will not directly affect basic research, but it is slowing down the translation of research discoveries into routine clinical care.

Moving forward will require looking at the bigger picture. There may not be rigorous clinical trial data proving that sequencing this or that gene will directly benefit the patient. But that should not prevent the understanding that this methodology can save health care dollars, and will ultimately allow physicians to provide better care. Providing the evidence for this is very difficult, although there are many individuals who have devoted their careers to doing so.

**H&O** Is next-generation sequencing being increasingly integrated into clinical trials?

**RP** Yes, absolutely. In both the academic community and the pharmaceutical community, researchers are aware that the drug pipelines are way behind the biomarker pipelines. In many clinical trials, the eligibility criteria now include genetic information. For many studies, the presence of a certain mutation is what determines whether a patient can enter a trial, not the tumor type. Describing cancer in terms of its cell of origin will not be disappearing any time soon, but more and more, our understanding of cancer as a genetic disease is shaping the way we research and treat this disease.

**Suggested Reading**


