Next-Generation Pathology

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Although the term “personalized medicine” has received a number of definitions over the years, it can simply be defined as the individualized detection of the risk of developing disease, typically by performing germ-line DNA sequencing and the prediction of response and avoidance of toxic effects from disease therapy through the use of “companion diagnostics.” In October 2010, a group of stakeholders from a wide variety of pathology societies, governmental organizations, and commercial groups gathered at the Banbury Conference Center, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, to discuss “genome-era pathology, precision diagnostics, and preemptive care.” A report of the proceedings of that conference by Tonellato et al entitled “A National Agenda for the Future of Pathology in Personalized Medicine” is published in this issue of the Journal. The report by Tonellato et al is described by the authors as a “call to action” for a national plan to change the nature of the practice of pathology and the education of medical students and pathology residents in the field of whole-genome sequencing (WGS) and clinical analysis. The authors contend that the rapid advances in DNA sequencing technology will rapidly enable the transition from the traditional dye terminator sequencing approaches featuring a “one gene at a time” and “hot spots only” approach to the massively parallel or next-generation techniques that enable multiplex testing of many genes in their entirety. Furthermore, the authors contend that it is of critical importance that pathologists become “owners” of these new genome-wide tests and lead this transition to more complex and precise prediction of disease risk and therapy response.

Next-Generation Comprehensive Genome Analysis

The first draft of the human genome sequence took more than 13 years to develop at a cost of approximately $3 billion. It is anticipated that, at current rates of technology advancement, a complete human genome sequence can now be generated by next-generation comprehensive genome analysis (NGS) technology in 5 to 8 days at a cost of approximately $2,000. NGS has the ability to fully sequence large numbers of genes (hundreds to thousands), all in a single test, and can simultaneously detect deletions, insertions, copy number alterations, translocations, and exome-wide base substitutions (including known “hot-spot mutations”) in all known disease-related genes. But NGS has significant challenges to overcome before it can become a widely used clinical test, particularly with respect to demands on expertise and infrastructure. Extensive computational expertise is required to bring NGS into clinical context, and a deep knowledge of pathology, pathobiology, and clinical medicine is required to generate truly useful, so-called clinically actionable reports to clinicians and treating physicians. Although it is anticipated that Clinical Laboratory Improvement Amendments–certified, laboratory-developed NGS tests will be commercially available for selecting treatment for patients with cancer in 2011, continuing advances in NGS technology are expected to lower the overall cost, shorten the turnaround time, increase the breadth of genome sequencing, and detect epigenetic markers and other important genomic parameters while becoming applicable to smaller and smaller specimens during the same period.
Roles for Pathologists in NGS-Based Personalized Medicine

In the report from the Banbury Conference Center, Tonellato and colleagues\(^1\) have responded to the anticipated impact of the explosive growth in gene sequence information on laboratory diagnostics and pathology practice by recommending the execution of 7 “Blue Dot” projects. All of these projects are designed to enhance awareness of pathologists and enable the introduction of whole-genome technologies (WGTs) into diagnostic settings such as cancer and pediatric developmental disorders. Although all 7 projects certainly have merit and are important to pathologists in training and in practice, they represent a wide range of opportunities and challenges. Project 1 is, without doubt, a “no-brainer.” The need to introduce NGS and WGT topics into medical student and pathology resident education is mandatory, and the authors are well on their way to leading this curriculum reform.

Similarly, having organized pathology compile a current listing of available tests as described in project 2 is definitely worthwhile. It is important that this list include all providers and not be restricted to hospital-based laboratories. The extreme high cost of entry to initiate clinical NGS and WGT with some sequencers now approaching list prices of $1 million will keep all but the most sophisticated and high-volume hospital-based clinical laboratories off of the test provider list. Of course, over time, this cost of entry could rapidly be reduced allowing smaller and less well-capitalized laboratories to enter this testing space.

The establishment of a shared clinical database or “repository” of genomic information, as outlined in project 3, using appropriate individual patient protection is emphasized by the group with good reason. Pathologists can fulfill major roles in achieving this goal whether their own laboratories directly perform NGS or not. In particular, when disease is sequenced, such as will be the case for cancer cells, pathologists need to weigh in on critical issues such as the need for a comparative sequencing of the patient’s germline DNA and whether other test formats such as messenger RNA and microRNA profiling, epigenomic status determination, and traditional on-slide testing (immunohistochemical analysis and fluorescence in situ hybridization) are also needed to personalize the patient’s care.

Project 4 is highly problematic given the rapid development of WGS testing in cancer and the likely introduction of commercial NGS later in 2011. The critical goal of this approach is the generation of “actionable” discoveries in the sequences that will clearly indicate a specific treatment pathway. For example, for the cancer genome, NGS may reveal that sequence abnormalities such as increased \(\text{HER2}\) gene copy number, \(\text{EGFR}\) and \(\text{CKIT}\) activating mutations, \(\text{BRAF}\) V600E mutations, and \(\text{EML4-ALK}\) translocations occur in small percentages in unexpected oncology settings not previously known about that could conceivably alter the patient’s treatment or lead to entry into clinical trials for targeted therapies. There simply may not be enough time for pathologists to compile WGS classifications as described by Tonellato et al\(^1\) in project 4 before widespread clinical testing has already started.

Project 5 is clearly well within the “sweet spot” for pathology. The accreditation and regulatory guidelines for WGS diagnostics must have major input and control by pathologists and organized pathology. Although the handling issues of cheek swabs and blood collections for DNA extraction are very important, for diseased tissue screening, it is fully expected that formalin-fixed, paraffin-embedded specimens will be the sample sources for NGS. Pathologists understand the critically important issues such as the impact of preanalytic variables, such as preprocessing ischemia, the nature and duration of tissue fixation, and the impact of tissue processing, on DNA extraction and sequencing accuracy. In addition, by using their morphologic skills, pathologists must study the routine histologic features of diseased tissues such as cancers to decide such issues as the following: (1) Will there be enough DNA in the tissue sample after extraction to load the sequencer? (2) Does enrichment have to take place to prevent dilution of tumoral DNA by adjacent benign tissues? (3) What block should be used to generate the DNA, eg, primary tumor or metastatic lesion, if available? (4) What part of a primary tumor should be used for sequencing, eg, the surface, the central region, the most invasive or “leading” edge. (5) Most important, what is the best way to handle needle biopsy specimens and fine-needle aspiration samples to generate enough DNA for sequencing?

Project 6 is certainly exciting to anticipate and, if the authors can see this through, will create a whole new pathology practice style with far greater direct clinical consultation activities. Regardless of whether a pathologist has directly overseen the performance of the NGS “test” and regardless of whether it was done in-house or referred to a commercial or academic reference laboratory, a local pathologist needs to use the training received in project 1 to bring the information to the relevant clinical specialists (eg, medical oncologists) in an actionable format. An NGS result has the potential to provide hundreds of data points, many of which may not have known relationships for the clinical care of the patient. No matter how well the NGS report is written by the test provider, pathologists must be able to help “interpret” the report and emphasize the mutations, deletions, translocations, and copy number changes that are important for immediate personalization of the patient’s care, whether it be available therapy selection or referral to a clinical trial.

The final project, project 7, referring to reimbursement issues, can impact pathology practice in a variety of ways. Certainly, if NGS is performed by a pathologist’s laboratory, this will be a critical issue needing input from the performing laboratories. But for pathologists working in facilities that refer
the NGS to a reference laboratory, there are still concerns about reimbursement for the sample preparation, such as the proper charging for Current Procedural Terminology code 88387.

In his essay, “The Law of Accelerating Returns,” Kurzweil argues that the speed, cost-effectiveness, or overall “power” of a process increases exponentially over time. Essentially this description applies exactly to the impact of NGS technology on the rate of DNA sequencing: the rate of exponential growth itself has grown exponentially. When the human genome scan was completed in 1987, experts in the field argued that it would take thousands of years to finish, but it came in slightly ahead of schedule. Now the entire project can be repeated in a few days at a fraction of the cost. This type of rapidly accelerating technology breakthrough will change what pathology is and what pathologists do in the future. Revolutionary approaches in high-resolution imaging show potential to perform in vivo microscopy and make “surgical pathologic diagnoses” without the surgery (or biopsy procedure). Nanoscale technologies are focusing on developing complex microscopic-sized clinical laboratories that can be injected into the patient and take their “readings” as they pass through the tissue or organ of interest. In their call for pathology education and pathology practice leadership to seek ownership of the WGS and NGS technologies, Tonellato and colleagues have found an excellent location to recommend the placement of our flag in the territory of what may well become a cornerstone of the pathology of the future.

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References


