Next-generation sequencing (NGS) technology has been moving at an arguably unprecedented pace compared with any other molecular genetics technology to have previously hit the scene. In both the clinical and research settings, this technology demonstrates great appeal because of its tremendous potential to affect medicine via its capacity to more thoroughly interrogate the genome at a fraction of the cost of previous technologies. Two recent articles in the journal *Nature* highlight the new and changing face of clinical testing and human genetics research. The first, a News in Focus article, discusses the fast-moving pace of NGS technology and some of the benefits and challenges to providing this testing clinically (1). In the second, a commentary article, the author points out the many possibilities for error in human genetics research, and proposes that these testing processes be moved into clinical laboratories (2). A tie-in between these 2 articles is the broad application of NGS to benefit both research participants and clinical patients.

Most current clinical NGS tests involve targeted panels of genes, but this testing is now expanding into test offerings for whole-exome and whole-genome sequencing. As highlighted by the News in Focus article, one of the biggest concerns with NGS is determining which identified variants are related to the patient’s phenotype and therefore potentially clinically actionable (1). The relevance of most variation in the human genome is still not clearly understood, in part because there is no reference or “normal” human genome (due to the variation among unrelated individuals). Additionally, whole-exome and whole-genome sequencing may uncover tens of thousands and millions of variants, respectively, per patient. That presents a substantial burden to laboratories and clinicians to perform due diligence in determining the potential pathogenicity of identified variants. Now more than ever, informatics specialists play a crucial role in the clinical laboratory by providing tools and resources to analyze NGS data and annotate variants.

The large volume of sequencing data presents additional pre- and postanalytical challenges. Pretest counseling to determine a patient’s expectations, understanding, and needs is imperative, but it may require investing numerous hours with each patient to ensure that the appropriate level of counseling is achieved. Postanalytically, clinicians must be provided with concise, yet complete, interpretive reports, and they must be given the time and resources to properly deliver these results to their patients. Thus, identifying and expanding resources to provide thorough pre- and postanalytical patient care for NGS is not a trivial matter, not to mention the education and training required to ensure that the clinicians themselves are prepared to practice genomics medicine.

In the *Nature* commentary article, Lyon raises concerns about the various errors that can occur with human genetics research testing, which ultimately lead to the dilemma about providing research-based results that may affect clinical decisions (2). He proposes that the processes involved in human genetics research—from sample testing to genome sequencing—be moved into the clinical setting; however, that could present a substantial financial burden to researchers to provide funds to accomplish this goal.

Current clinical practice requires the confirmation of all NGS variants via Sanger sequencing before reporting results. Thus, impromptu processes of developing and validating novel Sanger assays for clinical use are becoming more streamlined than ever. Therefore, a proposal that would be an alternative to the one described above would be for research laboratories to perform the NGS analyses and then collaborate with CLIA laboratories to confirm results via Sanger sequencing. This mode of work flow would undoubtedly be more affordable than providing CLIA laboratory-based research NGS testing and confirmatory analysis from start to finish and would additionally deliver equally high-quality results that potentially could be placed in the patient’s medical record, if that is the goal.

In conclusion, these are certainly exciting times, because NGS is having a tremendous impact on research, medicine, and clinical care. There are already numerous reports on the use of NGS to identify novel genes associated with disease, as well as to provide benefit to patients, research participants, and their families. Nonetheless, NGS comes with its uncertainties and
recognized limitations of a higher order of magnitude than previously seen in the field, owing to the nature of the technology. Uncertainties in genetic testing are nothing new, however, and we certainly have the foresight and fortitude to develop tools to deal with these issues. Therefore, we must continue to move forward and work together to use this powerful and promising technology to the best of its ability to benefit medicine.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors' Disclosures or Potential Conflicts of Interest: No authors declared any potential conflicts of interest.

References

DOI: 10.1373/clinchem.2012.185314

Tackling Neglected Areas of Oncology with Provocative Questions
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In an era of low funding rates and tightening budgetary constraints, researchers are inevitably nudged toward taking a conservative approach and thus focus on safe research questions to which answers are likely to be found. This approach not only risks limiting the scope of conducted research, but also discourages thinking outside the box and tackling highly ambitious and not immediately obvious questions, or questions that have proven exceptionally difficult to address. Yet answering these types of questions may have very important ramifications for both clinical medicine and the advancement of scientific knowledge.

To combat these issues, the US National Cancer Institute (NCI) developed the Provocative Questions initiative, an innovative new program aimed at promoting research in neglected and unsolved areas of oncology (1). Through workshops held at NIH and other locations across the US, the NCI solicited perplexing questions in cancer research that need to be addressed, but would usually be hard pressed to receive substantial attention. The scope of this process was expanded through the NCI’s Provocative Questions website (2), where scientists from around the world could propose their own intriguing questions, comment on existing questions, and learn more about the Provocative Questions initiative.

The importance of the 24 most appealing questions, as well as the likelihood and the potential implications of answering them, are described in detail at the Provocative Questions website (2). Examples of these questions include:

1. Given the recent successes in cancer immunotherapy, can biomarkers or signatures be identified that can serve as predictors or surrogates of therapeutic efficacy?
2. How does the lifespan of an organism affect the molecular mechanisms of cancer development, and can we use our deepening knowledge of aging to enhance prevention or treatment of cancer?
3. What environmental factors change the risk of various cancers when people move from one geographic region to another?
4. Given the appearance of resistance in response to cell-killing therapies, can we extend survival by using approaches that keep tumors static?

The NCI provisionally set aside $15 million from the 2012 budget to fund the most powerful ideas for answering any of the 24 provocative questions in cancer research. The request for applications received a strong response, with each question catching the interest of several scientists. Although this response is perhaps not surprising in the current funding climate, it does imply that many re-