Perspective

Translating genomic information into clinical medicine: Lung cancer as a paradigm

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We are currently in an era of rapidly expanding knowledge about the genetic landscape and architectural blueprints of various cancers. These discoveries have led to a new taxonomy of malignant diseases based upon clinically relevant molecular alterations in addition to histology or tissue of origin. The new molecularly based classification holds the promise of rational rather than empiric approaches for the treatment of cancer patients. However, the accelerated pace of discovery and the expanding number of targeted anti-cancer therapies present a significant challenge for healthcare practitioners to remain informed and up-to-date on how to apply cutting-edge discoveries into daily clinical practice. In this Perspective, we use lung cancer as a paradigm to discuss challenges related to translating genomic information into the clinic, and we present one approach we took at Vanderbilt-Ingram Cancer Center to address these challenges.

Knowledge about genetic alterations that drive and sustain the growth of various cancers is exploding. Using lung cancer as a model, we describe evolving trends and challenges in leveraging genetic information to inform cancer care. We briefly review the history of chemotherapy and targeted therapies in lung cancer, with a focus on the current era of therapies targeting the protein products of driver mutations occurring in single oncogenes. We discuss challenges related to how tumors will be profiled, including issues related to who should perform testing and with what platform, who should pay for testing and how gene patents may affect costs, which tumors to profile and when, and the concept of companion diagnostics. We also discuss issues related to clinical interpretations of genomic information: how will they be reported to clinicians, in what format, and using what knowledge resources (Fig. 1). Finally, we present one approach that we took at the Vanderbilt-Ingram Cancer Center to address these issues for our personalized cancer medicine initiative.

Lung cancer

Lung cancer is the leading cause of cancer-related death in the United States (Siegel et al. 2011). The overall 5-yr survival for all stages is ~16%. Since at least 1968, when the international tumor-node-metastasis (TNM) staging system was officially adopted as the "international language" for diagnosis and treatment of lung cancer, patients' tumors have been classified primarily according to tumor histology (International Union Against Cancer 1968). Clinically, the two main subtypes of lung cancer include small cell lung cancer (SCLC; ~10% of cases) and non-small cell lung cancer (NSCLC; ~90% of cases). This Perspective will focus primarily on NSCLC, the major histologic subtype in which advances in the genomics of lung cancer have been made. NSCLC is further subdivided into adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. Unfortunately, more than half of patients with NSCLC are diagnosed at advanced stages, where treatment is not curative.

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Treatment of lung cancer with chemotherapy

Although lung cancer is a heterogeneous disease, the paradigm for the treatment of metastatic disease from the late 1970's to the mid-2000's was largely empiric, based upon observations made in clinical trials involving cytotoxic chemotherapies. The major classes of chemotherapies include alkylating agents (e.g., cisplatin), antimetabolites (gemcitabine), microtubule inhibitors (e.g., paclitaxel), topoisomerase inhibitors (e.g., etoposide), and cytotoxic antibiotics (e.g., doxorubicin). These agents generally kill cells that divide rapidly, one of the key properties of most cancer cells. The dogma by the late 1990's was that all patients with metastatic NSCLC should receive "modern platinum-based chemotherapy doublets" (i.e., carboplatin plus an agent such as paclitaxel or gemcitabine), regardless of histology (Schiller et al. 2002). With this "one-size-fits all" approach, the therapeutic efficacy of treatment reached a plateau. A landmark trial in which ~1200 patients with advanced NSCLC were randomly assigned to one of four platinum-based chemotherapy regimens showed no significant advantage of any one regimen over the others in terms of overall survival (~8 mo) (Schiller et al. 2002). The addition to platinum-based doublets of bevacizumab, a monoclonal antibody against vascular endothelial growth factor (a major regulator of angiogenesis in normal and malignant tissue), led to only a 2-mo survival benefit (12 mo versus 10 mo) in one study (Sandler et al. 2006) but not another (Reck et al. 2009) and was associated with an increased risk of treatment-related deaths (Sandler et al. 2006). Despite multiple efforts to identify molecular predictors of response to cytotoxic chemotherapy, none are used routinely in clinical practice (Andrews et al. 2011).

Treatment of lung cancer with targeted therapies in the beginning of the genomic era: The new taxonomy of single gene driver mutations

The first draft of the human genome was published in 2001 (Lander et al. 2001; Venter et al. 2001). Although a handful of genes (i.e., *KRAS, NRAS, PTEN*) were shown to be mutated in lung cancers prior to 2001, most tumor-specific gene alterations have been identified since that time (Fig. 2). With the advent of whole exome, whole genome, and whole transcriptome sequencing, the pace of discovery is accelerating.

Table 1. Molecular subsets of NSCLC defined by "driver" mutations

Gene	Frequency	References
AKT1	≤1%	Bleeker et al. 2008 Do et al. 2008 Malanga et al. 2008
ALK	3%–7%	Soda et al. 2007 Koivunen et al. 2008 Kwak et al. 2010
BRAF	1%–3%	Pratilas et al. 2008 Lee et al. 2010 Paik et al. 2011
EGFR	10%–30%	Lynch et al. 2004 Paez et al. 2004 Pao et al. 2004
FGFR1	20%	Weiss et al. 2010 Dutt et al. 2011
HER2	2%–5%	Sonobe et al. 2006 Lee et al. 2010
KRAS	15%–30%	Tsao et al. 2006 Riely et al. 2008
MEK1	1%	Marks et al. 2008
NRAS	1%	Brose et al. 2002 Ding et al. 2008
PIK3CA	1%–3%	Kawano et al. 2006 Lee et al. 2010
RET	1%	Kohno et al. 2012 Takeuchi et al. 2012 Lipson et al. 2012 Ju et al. 2012
ROS1	1%	Bergethon et al. 2012

kinase inhibitor, crizotinib (Kwak et al. 2010). In contrast, typical response rates in the setting of pretreated lung cancer are typically around 10% (Hanna et al. 2004). Patients with *ALK* fusion-positive lung cancer do not respond to treatment with EGFR TKIs (Shaw et al. 2009). Similarly, *ROS1* fusion-positive tumors are also highly sensitive to crizotinib (touted as an ALK TKI, but with activity also against ROS1 and MET) (Bergethon et al. 2012). In addition, *ERBB2* (*HER2*) mutant tumors are responsive to HER2 TKIs (De Greve et al. 2012), and *BRAF* mutant tumors may be responsive to the mutant-specific BRAF inhibitor, vemurafenib (Gautschi et al. 2012).

As a result of these compelling data, multiplex mutational profiling of lung tumors is now part of accepted standard clinical and pathology practice at locations throughout the US (Pao et al. 2009; Dias-Santagata et al. 2010; Sequist et al. 2011; Su et al. 2011). Such "first-generation" profiling of about 10 genes implicated in lung-cancer pathogenesis will detect mutations in roughly half of unselected NSCLCs and nearly 90% of lung adenocarcinomas from East Asian never smokers (Sun et al. 2010; Li et al. 2011).

Maximizing the potential of genetically informed cancer medicine

The explosion of genomic information offers great promise toward an era of "precision" or genetically informed cancer medicine, where patients are treated rationally according to the genetic makeup of their individual tumors rather than empirically based upon histology. We are just at the beginning of this paradigm shift, especially because translation of basic scientific findings to the clinic often takes time and education for all involved. In addition, the knowledge explosion presents many challenges (Fig. 1). Below we discuss many of the issues that remain to be addressed and our preliminary experience at the Vanderbilt-Ingram Cancer Center in solving them.

How will tumors be profiled?

DNA sequencing technology is undergoing a revolution, and advances in massively parallel technologies have dramatically reduced the cost of sequencing (Macconaill and Garraway 2010). These developments imply that comprehensive tumor genome profiling will not only occur as part of pilot programs (Roychowdhury et al. 2011) but will also become widely adopted in the future. To be clinically applicable, such profiling should be performed in the appropriate settings, i.e., in laboratories that are compliant with the Clinical Laboratory Improvement Amendment (CLIA) of 1988 administered by the Center for Medicare Services (CMS) and in accord with guidelines set forth by the College of American Pathologists (CAP) (for details, see Febbo et al. 2011). Presumably, most assays will be performed using formalin-fixed paraffin-embedded tissues and not fresh frozen tissues, since in the vast majority of patients with metastatic disease, only the former is available.

Who will perform the testing, local hospitals or reference laboratories?

Some local and many academic hospitals are able to perform a limited number of molecular and cytogenetic tests on a select number of cancer genes using technologies that have been widely available in CLIA labs for many years. However, the technology platforms for high throughput tumor gene mutation testing are changing every year. How will molecular diagnostics laboratories keep up with the cost of replacing their technology and validating the new assays every few years? Can each local hospital afford to develop/perform such assays on its own, or will such assays need to be outsourced because of their inherent complexity? Until the "landscape" is settled, reference laboratories and only a handful of well-endowed institutions are likely to be able to offer comprehensive tumor profiling for patients. Perhaps even more importantly, there are a limited number of trained molecular pathologists worldwide who can run such labs. The training of future pathologists will need to change to accommodate the rise in demand for molecular testing.

Who will pay for the molecular testing?

The cost of current molecular diagnostic assays can vary from hundreds of dollars (for single gene sequencing) to thousands of dollars (for multigene profiling or fluorescent in situ hybridization). The costs of such tests are reimbursable by insurers, but at some point, as the "$1000 genome" becomes a reality (Macconaill and Garraway 2010), the cost of comprehensive tumor profiling will actually be cheaper and more practical than individual gene tests. When that time comes, it will be more cost effective on a societal level for patients diagnosed with cancer to undergo routine comprehensive tumor profiling at various stages of treatment, provided that the use of expensive therapies is restricted to only those most likely to benefit.

How will gene patent licensing be addressed?

In 2005, it was estimated that nearly 20% of human genes were explicitly claimed as U.S. intellectual property (Jensen and Murray 2005). Many important cancer genes are covered by patents, so not everyone can legally analyze them for commercial purposes without proper licenses (Kean 2011). In fact, investigating all of the relevant patent claims (issued and pending) for possible infringement on the testing of about 100 cancer genes was estimated in

2011 to cost at least $35 million (Kean 2011). Creative solutions among all stakeholders, including lawyers, businesspeople, and policymakers, will need to be made to pave the way forward. Fortunately, recent arguments have been made that perhaps these claims have been overstated and there appears to be little evidence that would support an assumption that gene patents pose a substantial impediment to technologies like whole-genome sequencing (Holman 2012).

What sequencing platform should be used?

How much average gene depth and coverage are appropriate for profiling? Is variable coverage across key genes acceptable? Does the whole exome need to be done, the whole genome, or the whole transcriptome? Or should just "cancer genes" be examined? Importantly, can the test and the interpretation be performed in a clinically relevant timeframe, i.e., within 1 to 2 wk of the first clinic visit, so that patients with metastatic disease can start treatment as soon as possible? One current "second-generation" approach involves targeted capture and resequencing of about 200 genes implicated in cancer with high coverage (Lipson et al. 2012). This particular platform is attractive because it detects all known genomic alterations that could have relevance, i.e., point mutations, insertions, deletions, rearrangements, and copy-number alterations. Moreover, the assay is performed in a CLIA-certified laboratory with a quick turnaround time.

What about companion diagnostics?

According to the US Food and Drug Administration, a companion diagnostic device is an in vitro diagnostic (IVD) device that provides information that is essential for the safe and effective use of a corresponding therapeutic product. Examples include an *ALK* fluorescence in situ hybridization (FISH) test to detect ALK gene rearrangements for the use of crizotinib in lung cancer and an allele-specific PCR-based test to detect $BRAF^{V600E}$ mutations for the

observation may not indicate that it should be used for routine clinical care. Clinical utility implies that high-level evidence shows that use of the marker improves patient outcome sufficiently to justify its incorporation into routine clinical care. As mutations are uncovered, we will eventually know all of the ones associated with clinical utility, i.e., that can help prioritize therapy. Until that time, however, we will need to be flexible and inclusive, but cognizant that most of the mutations identified will be of unknown significance. One approach that many places are adopting involves the reporting of clinically actionable mutations, with statements regarding (1) whether a mutation has been linked to an FDA-approved drug within the specific disease being tested, (2) whether a mutation has been linked to an FDA-approved drug within a different disease from that being tested (since effective targeted treatment in one disease does not always translate to another), (3) whether a mutation has been linked to a non-FDA approved drug in preclinical models or early phase clinical trials, and (4) whether a mutation has not been linked to any drug.

How will tumor mutation results be reported?

Current methods for reporting tumor gene mutation testing results for clinical use have several limitations with respect to report content and format. Today, most molecular diagnostics laboratories perform tumor gene mutation testing on one or two genes at a time, and only test for the few most common mutations within that gene. The report content consists of a full-page text description of each gene tested. The report contains static information regarding the testing technique and the clinical significance of the gene mutation detected with respect to drug therapies for a particular disease (i.e., "tumors with *EGFR* exon 19 deletion are sensitive to EGFR TKI's in non-small cell lung cancer").

While reports typically contain one or two references to support these types of high-level statements regarding drug sensitivity, they may not have the most up-to-date information. The references are essential to clinicians in that they provide important information regarding the appropriate clinical context for use of these drugs (e.g., in the metastatic or adjuvant setting). This level of detail assists the treating physician in making actionable treatment decisions taking into account the tumor gene mutation results. Even when the information is most current, it is only relevant for that moment in time when the report is created. Six months later, when the patient's tumor has progressed and a new treatment is considered, the interpretation in the original report may no longer be up-to-date given the pace at which new knowledge in this area is emerging. How will providers and patients remain updated regarding changes in the interpretation of results as new evidence emerges?

In addition, these reports lack information regarding tumor/gene mutation-directed clinical trials, one of the main actionable treatment decisions that can result from this type of molecular testing today. However, clinical trial availability changes even more rapidly than the clinical evidence. Even when open clinical trials are included in the interpretive report at the time it is generated, how will providers and patients become notified when new trials are available that may be relevant to the patient's care?

Furthermore, the current approach of reporting the significance of one gene at a time does not address the need to account for how multiple genes affect the sensitivity of a particular drug or drug regimen. This is in part due to the gene-oriented view of most reports as opposed to a drug-oriented view where the results of multiple genes can be taken into account simultaneously. This is a difficult challenge to solve given the current level of clinical evidence; however, it will become more and more important as new targets and new therapies emerge.

There are also several limitations with respect to the format of the reports themselves. Free-text reports are typically scanned into the electronic health record (EHR) or transmitted via HL7 messaging from the laboratory information system as text documents. Free-text reports and image files are not computable with respect to enabling downstream clinical decision support for treating physicians. However, there are a limited number of laboratory information systems that have adequate structured representation of genetic testing results. Organizations such as HL7 are working in conjunction with molecular diagnostic and testing societies to develop an information model to adequately represent genetic testing results. However, the challenges with respect to next-generation sequencing technologies are great in this regard.

Finally, the interpretive reports are static documents that are only relevant at a moment in time, a requirement for medical-legal purposes. While the results of the mutation testing for a given date will not change (e.g., EGFR T790M mutation detected on specimen from January 15, 2011), the interpretation of the clinical significance of the results will change over time. Thus, an opportunity exists to separate the static from the dynamic components of molecular diagnostic reports. The current approach of one report for one gene does not scale when comprehensively reporting the results for multiple genes and mutations. Thus, multiparameter data visualization and presentation represent another significant challenge.

How will tumor mutation results be used in clinical decision support systems (CDSSs)?

Beyond the traditional approach of providing decision support through interpretive reports, opportunities exist to utilize tumor gene mutation testing results directly in clinical decision support systems for cancer treatment plan selection. CDSSs have recently been developed to support pathway and guideline compliance (Neubauer et al. 2010). These tools may be standalone applications or integrated directly into electronic health records. They provide recommendations for treatment selection based on multiple tumor and patient features. Clinical practice guidelines for ASCO and the National Cancer Comprehensive Network (NCCN) already recommend EGFR testing in lung cancer as standard of care, but they provide little support for variant level interpretation of results to guide treatment. It is anticipated that CDSSs will utilize these new tumor features in their recommendations for treatment prioritization, but exactly how remains to be seen.

How will knowledge bases that catalog the associations between mutations and clinical outcomes be maintained and funded?

From the above discussion, it is clear that knowledge bases are needed to support clinicians, molecular pathologists, and patients in understanding the evolving clinical significance of tumor gene mutations. Who will fund these knowledge bases and who will maintain them? With some mutations occurring at a frequency of <1%, clinical trials cannot be expected to answer every question regarding the significance of rare mutations. Similarly, observational cohort studies and case reports in the literature can provide a starting point for knowledge extraction, but also will not provide sufficient power to account for rare mutations and multiple simultaneous mutations. Large databases of patient level cases from

multiple institutions will be needed to aggregate knowledge sufficient for discovery and interpretation of clinical significance. We have started one such database for *EGFR* mutations in lung cancer (Horn et al. 2011).

Implementation of genome directed cancer treatment at Vanderbilt

The Vanderbilt-Ingram Cancer Center was faced with the challenges discussed above when we implemented "first-generation" (SNaPshot-based) tumor gene mutation testing for NSCLC in our CLIA-certified molecular diagnostics lab in July 2010 (Su et al. 2011). Initially, we needed to determine which tumors to test and when. We chose NSCLC and melanoma because they had actionable mutations as part of standard of care and active clinical trials. Tumors are tested at diagnosis and at time of rebiopsy after disease recurrence or progression. We took a practical and economic approach to selection of the molecular diagnostic testing platform in choosing to use an ABI SNaPshot platform that was already in use in our CLIA certified diagnostic lab. Given that a limited number of mutations could be tested simultaneously using this platform (~40), we chose to create disease-specific testing panels for lung cancer and melanoma. We selected variants to test that were prevalent in at least 1% of tumors in each respective disease. This helped maximize the likelihood of a mutation being detected using this assay.

Faced with reporting over 40 different mutations in nine genes for lung cancer, we needed an alternate solution to the traditional one-gene one-report approach to reporting. To address the above issues, we made the key decision to decouple reporting of the tumor gene mutation testing results from the interpretation of their clinical significance. Tumor gene mutation results are now reported as discrete data elements in the laboratory information system. Each gene mutation in the panel is coded and reported as "Detected" or "Not Detected" which makes this information computable. The results are then transmitted to the electronic health record and visualized in multiple ways that aggregate and abstract the results to enable rapid review of a large amount of information (Fig. 3). Finally, individual results are linked to a knowledge base called "My Cancer Genome" that provides information on the clinical significance of specific gene mutations for a particular disease including information on gene-associated clinical trials open at Vanderbilt and worldwide. The information in My Cancer Genome is continuously updated, creating a dynamic interpretation for clinicians who may review the results several months later as the patient's state changes. This combination of reporting static testing results as discrete data, abstract data visualization, and links to dynamic knowledge services has provided an initial scalable solution at our institution. In addition to being integrated into Vanderbilt's EHR, My Cancer Genome is freely available online (www.mycancergenome.org) for public use.

Conclusion and future directions

Antimicrobial testing today is taken for granted as a routine part of medicine, but history suggests that it actually took a long time to become standardized. In 1928, Alexander Fleming discovered

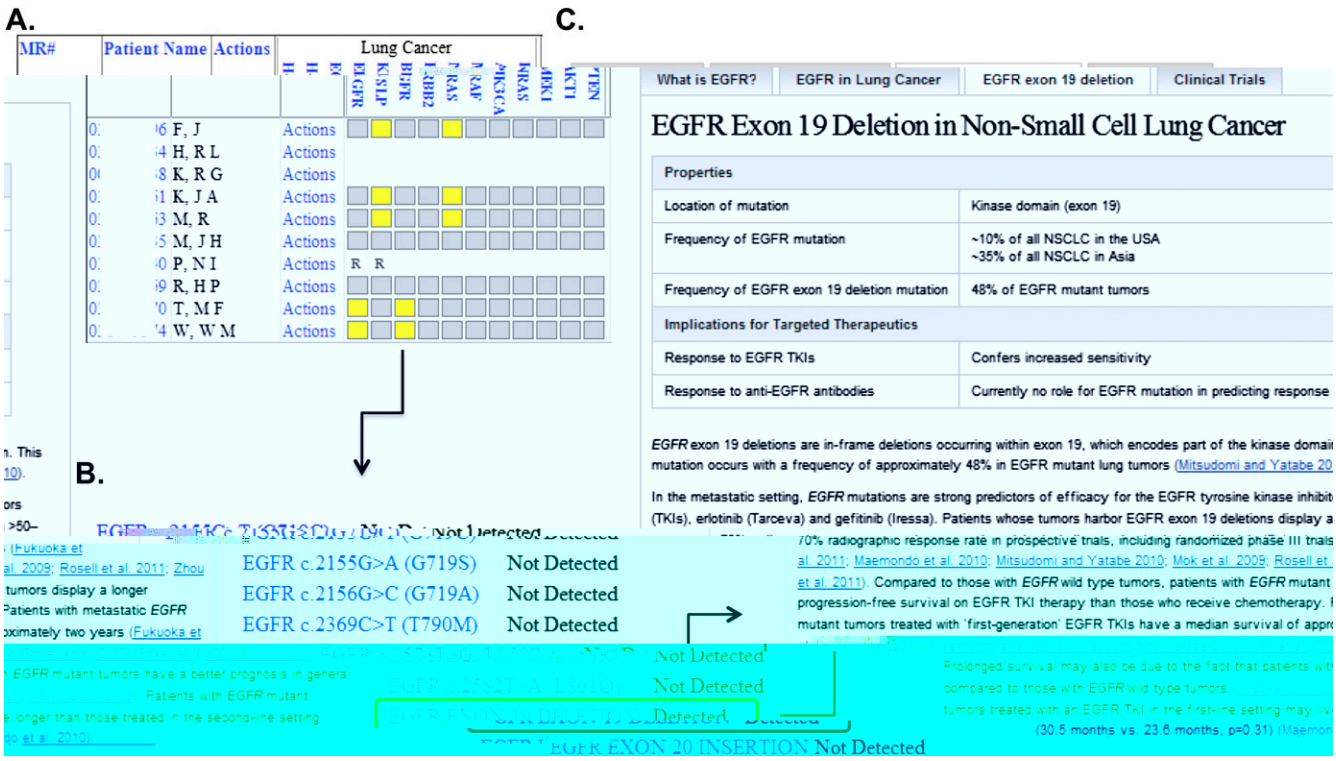

Figure 3. Screenshot visualizing tumor gene mutation results in the electronic health record linked to decision support. (*A*) In the provider dashboard, patients are listed in separate rows with their respective gene mutation results shown as colored indicators in each column (yellow: mutation detected; gray: mutation not detected). "H-GFR" and "H-SLP" refer to the specific diagnostic tests performed, as named by the molecular diagnostic lab, while the remaining nine columns refer to the specific genes assessed for mutations. The user clicks on a specific gene indicator to view the specific gene mutation results shown in *B*. Gene mutations are reported using the REFSEQ nomenclature with results reported as Detected or Not Detected. The user clicks on the specific gene mutation and is taken to *C*. Summary of the clinical significance for the specific gene mutation in the patient's specific cancer diagnosis.

penicillin, marking the start of modern antibiotics. In the same decade, he made a contribution to modern antibiotic susceptibility testing by developing a forerunner of contemporary minimum inhibitory concentration methodology. Diffusion methods of antibiotic susceptibility testing were developed further in the ensuing decades, but it was not until 1975, when a specific method (the Kirby-Bauer disc diffusion technique) became the basis for a routine standardized national method accredited by the National Committee for Laboratory Standards (Wheat 2001). Such antibiotic susceptibility testing reports are the staple of medicine today, allowing physicians to make rational and effective choices regarding the use of antibiotics to treat infections arising from diverse origins (e.g., gram-positive cocci, gram-negative rods, etc.)

Analogously, decades of research designed to elucidate the genetic basis of cancers have now revealed multiple clinically relevant molecular subsets of disease. These discoveries are leading to a new taxonomy of cancer, which holds the promise of rational rather than empiric approaches for treatment. Lung cancer currently represents one of the best paradigms for this new shift. Tumors can now already be tested for multiple different "driver" mutations at a time, helping to prioritize the use of targeted therapies most likely to benefit individual patients. However, we are just at the beginning of this new paradigm. Great efforts still need to be made to overcome the many challenges currently facing the field. When comprehensive mutational profiling becomes efficient, cost-effective, and easily interpretable by practicing clinicians, we are confident that just like antibiotic susceptibility testing, it will become a part of routine cancer care.

Competing interest statement

William Pao reports consulting for MolecularMD, AstraZeneca, Bristol-Myers Squibb, Symphony Evolution, and Clovis Oncology. His lab has received research funding from Enzon, Xcovery, AstraZeneca, and Symphogen. Rights to EGFR T790M testing were licensed on his behalf and others to MolecularMD by MSKCC. The other authors have no conflicts to disclose.

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