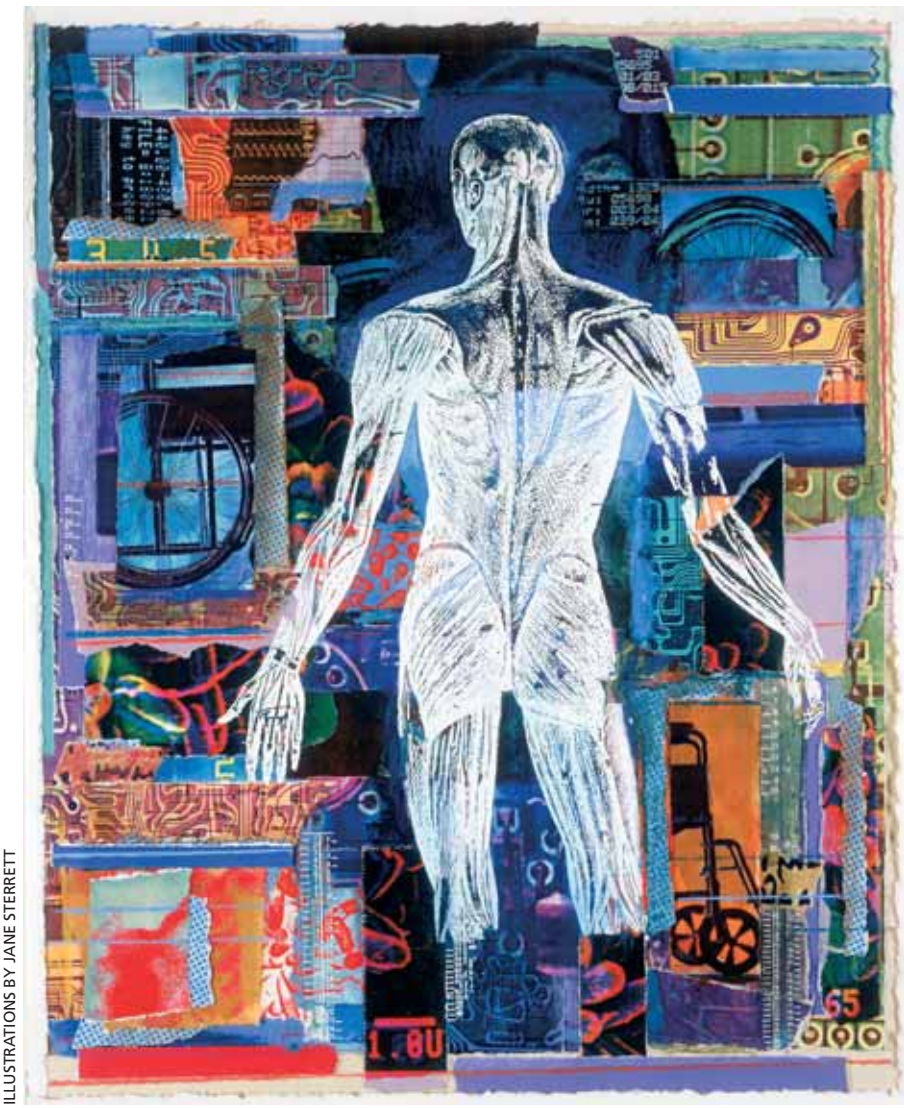
Cancer Biomarkers
Where Medicine, Business, And Public Policy Intersect

Molecular biomarkers are emerging as key indices for the management of patients with cancer and other significant diseases, but their potential has yet to be fully realized. Is there enough collaboration in place to optimize this care revolution?

By Chantell L. Wilson, PhD, Stephanie Schultz, PhD, and Scott A. Waldman, MD, PhD

In 2005, cancer exceeded cardiovascular disease as the leading cause of mortality in people younger than age 85 in the United States (Dalton 2006). In 2006, approximately 500,000 U.S. cancer patients died from their disease. Moreover, beyond cardiovascular and infectious diseases, cancer is the third leading cause of disease burden worldwide. Advances in our understanding of how biological processes interact — thus enabling such technologies as genomics, proteomics, and metabolomics — combined with drug discovery and development, high-throughput screening, and *in silico* drug development, place science on the threshold of innovative cancer management. This innovation is predicated on the development of biomarkers that enable cancer prevention, diagnosis, and treatment



ILLUSTRATIONS BY JANE STERRETT

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in both individual patients and in general populations.

Biomarkers are characteristics that can be objectively measured and evaluated. They provide information about normal or pathophysiological processes to detect or define disease progression or to predict or quantify therapeutic responses. Traditional biomarkers have encompassed surrogate physiological measurements (such as heart rate, blood pressure, and performance status), imaging (such as chest X-rays and mammograms), and individual protein molecules (such as prostate-specific antigen [PSA] and carcinoembryonic antigen [CEA]).

Mapping the human genome in conjunction with rapid nucleic acid and protein analytic technologies have brought a new generation of molecular biomarker technologies within reach. These advancements include single nucleotide polymorphism (SNP) analysis, genomic and proteomic profiling, epigenetic profiling, and gene expression profiling. They carry the promise of increased disease-specific sensitivity and specificity coupled with higher dimensional complexity to provide a greater level of individualized disease management.

The potential of the molecular revolution has stimulated a new generation of biotechnology entrepreneurs who are attempting to capitalize on the inherent importance of biomarkers to both individualized and population-based medicine. That potential, however, has yet to be fully realized either clinically or commercially, reflecting the asynchronous development of discovery technologies and paradigms for their analytic validation, clinical qualification, and application. This asynchrony and the associated paucity of validation of highly complex biomarkers have engendered previously unrecognized issues surrounding approval and marketing by regulatory agencies. The evolution in regulation and the emergence of requirements for robust analytic validation and clinical qualification, along with the attendant patient- and capital-intensive resources necessary to support these activities, have resulted in the development of new partnerships. Federal agencies, academia, and the pharmaceutical and biotechnology industries are now optimally poised to explore the full potential of molecular biomarkers.

INDIVIDUALIZED MEDICINE

The individualization of disease management is predicated on developing biomarkers that subserve specific clinical domains. *Preventive biomarkers* prospectively identify individuals at increased risk for developing disease. For example, patients with mutations in the genes encoding BRCA1 and BRCA2 are at risk for developing breast and ovarian cancer. Identification of these mutations mandates aggressive disease surveillance and genetic counseling for risk reduction in a patient's extended family, and may suggest prophylactic mastectomy for disease prevention (Meijers-Heijboer 2001). *Diagnostic biomarkers* identify the presence of disease at the earliest stage, before clinical manifestation. Serum PSA discovers patients who might harbor occult prostate cancer (Sidransky 2002), genomic stool screening identifies those with subclinical colon cancer (Imperiale 2004), and mammography seeks out occult breast cancer. Prognostic biomarkers stratify the risk of disease progression in patients undergoing definitive therapy. Gene mutation profiling in patients with estrogen receptor-positive lymph node-negative breast cancer identifies those at increased risk for developing recurrent disease (Paik 2004), and the loss of heterozygosity of specific genes or microsatellite stability identifies patients with lymph node-negative colon cancer who are at an increased risk for developing recurrent disease (Sidransky 2002).

Predictive biomarkers identify patients who are most likely to respond to a specific therapy. Quantification of HER2 expression identifies patients with breast cancer who overexpress this receptor and thus are likely to be particularly responsive to treatment with humanized monoclonal antibodies to HER2 (Wilson 2006). Therapeutic biomarkers provide a quantifiable measure of therapy response in patients undergoing treatment. Evaluation of minimal residual disease in patients with chronic myelogenous leukemia (CML), employing polymerase chain reaction (PCR) technology to detect the Philadelphia chromosome, quantifies the efficacy of therapy (Faderl 2004). Lastly, biomarkers identify patients at risk for the development of adverse reactions to specific therapeutics. Individuals with particular mutations in the catabolic enzyme thiopurine methyltransferase do not efficiently metabolize mercaptopurines, which can induce life-threatening myelosuppression in those patients treated for leukemia

(Marshall 2003). Similarly, patients with mutations in one isoform of the detoxifying enzyme uridine diphosphate glucuronosyltransferase are slow metabolizers of irinotecan (Camptosar) and can develop severe diarrhea and neutropenia in the absence of dose adjustments (Iyer 1998).

TECHNOLOGICAL ADVANCES

Motivated by the concerted effort to define the human genome, the creation of rapid analytic technologies for evaluating nucleic acids and proteins has provided the technological "push" for the development of molecular cancer biomarkers. In contrast, conceptual advances in elucidating the molecular mechanisms underlying tumorigenesis and the evolving concepts of tumor suppressors and oncogenes have yielded diverse and complex targets to satisfy clinical needs for the individualization of medical management, providing the associated "pull" for biomarker development. Initially, molecular cancer biomarkers evolved in the model of classical protein markers, like PSA in prostate cancer and CEA in colon cancer, and early genetic markers, like the Philadelphia chromosome for CML, as single elements related to the presence of disease. These new molecular markers emanated from discovery efforts that focused on the molecular basis of cancer, defining mutations in individual genes causally related to tumorigenesis. Their clinical application was potentiated by the development of rapid nucleic acid sequencing technology coupled with mutation-specific PCR for high-throughput analyses. Individual prognostic markers employed to assess cancer risk include mutations in adenomatous polyposis coli, associated with development of familial adenomatous polyposis and colorectal cancer, and those in DNA mismatch repair genes MLH1 and MSH2, associated with microsatellite instability and hereditary non-polyposis colon cancer (Sidransky 2002, Matloff 2004). Similarly, the detection of viral genes, including the human papilloma virus and the Epstein-Barr virus, can assess risk for cervical and nasopharyngeal cancer, respectively (Sidransky 2002). Prognostic information also can be derived from defining mutations in key signaling mechanisms dysregulated in tumors, including mutations in tumor protein 53 and Ras (Sidransky 2002).

Ultimately, the value of a biomarker to patient management must be qualified in an appropriately designed clinical trial with key definitive endpoints.

These initial linear approaches using single-molecule biomarkers have dramatically evolved to capture the richness of biosystemwide changes underlying pathophysiology. In its simplest form, panels of genetic markers and their disease-specific mutations are catalogued, and their cumulative prognostic or predictive value are established. Beyond panels of individual genes, the entire transcriptome can be assessed, distinguishing tumors from normal tissues or from those tumors with different risk profiles. Distinct patterns of gene expression in tumors and normal tissues have been elucidated in the breast and colon (Sjoblom 2006), and gene expression profiling can stratify risk in patients with lymphoma (Glas 2005). Similar approaches are being examined that employ patterns of disease-specific SNPs and epigenetic changes associated with DNA methylation (Sidransky 2002). Most recently, profiling the serum proteome through the use of mass spectrometry has distinguished patients with ovarian cancer (Petricoin 2002).

UNMET PROMISE

Although molecular biomarkers represent the envisioned future for individualized medicine, their potential has yet to be realized, reflecting issues of technique, study design, and pathophysiology (Dalton 2006, Wilson 2006). The molecular technologies from which these markers emanate have been prolific as discovery engines, but have not been systematically transitioned to generate robust assay performance consistent with requirements for routine clinical laboratories in the form of analytic validation, and defined disease management value in the form of clinical qualification. In that context, it is not unusual for biomarkers to be assessed by using so-called home brew assays in individual laboratories that have not undergone rigorous analytic validation to define performance metrics, such as reproducibility, sensitivity, and precision (Dalton 2006, Hudson 2006b). Additionally, molecular analytes may be evaluated

using different technical platforms whose performances have not been cross-validated. The absence of assay performance standards that reflect rigorous analytic validation and standardization across laboratories and platforms underlies issues of irreproducibility (Dalton 2006).

Additionally, quantitative and qualitative relationships between analytes and disease management have not undergone rigorous clinical qualification, and the evidentiary proof linking a biomarker with biology and clinical endpoints may not be readily available (Wagner 2007, Williams 2006). These relationships, which describe the marker's clinical utility, should be assessed in appropriately designed and powered prospective, blinded, randomized clinical trials, then validated in follow-up trials. Without this rigorous approach, a biomarker's clinical value can be overestimated, reflecting bias and chance resulting from overfitting. This occurs when statistical models are employed to fit too many predictive variables to an insufficient number of study participants (Wilson 2006).

A biomarker also may perform analytically and clinically, yet not alter the ultimate clinical outcome for patients (Miller 2006). A Japanese screening program for neuroblastoma that employed urinary biomarker testing successfully identified more cases of early-stage disease, but there were no changes in the number of late-stage cases or mortality (Honjo 2003). Indeed, the biomarker screening program identified cases that were normally indolent and, in fact, remained occult, ultimately regressing without causing morbidity and mortality. Here, biomarker screening provided overdiagnosis, creating pseudodisease that provided no real contribution to patient management (Miller 2006). This case illustrates that, ultimately, the value of a biomarker to patient management must be qualified in an appropriately designed clinical trial with key definitive endpoints.

BIOMARKER COMMERCIALIZATION

Molecular biomarkers have the potential to influence critical clinical decision making, substantially affecting healthcare economics. Preventive tests that screen for genetic mutations identify patients at risk for developing breast and colon cancer, who will thus become new customers to the healthcare system. Prognostic tests define the risk of breast cancer recurrence in those patients who may not benefit from expensive chemotherapy, while those tests that examine stool biomarkers distinguish patients who require further clinical evaluation with expensive endoscopic procedures. Predictive tests that examine the overexpression of HER2 receptors in breast tumors establish the suitability of patients to receive expensive monoclonal antibody therapy directed toward that target. The

impact on clinical outcomes and the associated allocation of limited healthcare dollars have been employed to justify high price points for molecular diagnostics along the lines of those traditionally seen for therapeutics (Licking 2006).

The emergence of molecular diagnostics as high-cost, high-profit products has therefore spurred biotechnology entrepreneurs and venture capitalists to launch a number of new companies that are focused on the development of molecular biomarkers in cancer and other diseases. Success depends on whether their products address substantial markets, and provide direction in critical clinical decision making regarding expensive, complex, or dangerous therapeutic interventions (Licking 2006). At stake is a $5 billion market that is growing at an annual rate of 25 percent (Pollack 2006).

Historically, the paradigm in the diagnostics business was to obtain approval from the U.S. Food and Drug Administration for the marketing of test kits that would then be sold to local clinical laboratories. In the new paradigm, molecular biomarker tests forgo FDA approval and distribution to local laboratories and instead are run in central laboratories (Licking 2006). Offering diagnostic tests from a central laboratory and abrogating the need for FDA approval permits a shorter and less costly development timeline from discovery to marketplace. However, savings in money and time can reflect the absence of definitive studies analytically validating and clinically qualifying the biomarker steps typically mandated by the FDA for marketing approval. Indeed, it is precisely this failure to provide definitive validation and qualification of the biomarker's value that has contributed to the relatively slow integration of molecular biomarkers into mainstream patient management paradigms (Dalton 2006, Williams 2006, Wilson 2006).

Abrogating the need for FDA approval permits may save money and time to commercialization, but this failure to provide definitive validation of the biomarker's value contributes to the relatively slow integration of biomarkers into mainstream patient paradigms.

TESTING AND OVERSIGHT

As molecular biomarkers rapidly emerge as key indices for managing patients with cancer and many other diseases, oversight and regulation of their safety and validity has lagged. Today, approximately 1,000 biomarkers are available as diagnostic tests, almost universally marketed as home brew tests without FDA approval, conducted in one central laboratory (Hudson 2006a, Hudson 2006b). The FDA does not regulate, certify, or provide oversight for these tests, nor does it guarantee their analytic validity or clinical qualification. Rather, decisions about the validity, utility, and clinical interpretation of tests are relegated to individual laboratory directors. In 1988, Congress enacted the Clinical Laboratory Improvement Amendments (CLIA), which required certification of any laboratory that performed testing on human specimens and reporting of patient-specific results. Under CLIA provisions, certification require laboratories to adhere to general requirements for quality control standards, personnel qualifications, and documentation and validation procedures. Moreover, laboratories that conduct high-complexity testing must enroll in a specialty area that provides for proficiency testing that directly correlates with the quality of offered testing services. In that regard, it is noteworthy that there is no specialty area identified for molecular and genetic testing, nor are there any specific quality control, personnel qualification, or proficiency testing requirements for these diagnostics (Hudson 2006b). The Centers for Medicare and Medicaid Services is generally responsible for the quality of clinical laboratory testing, and specifically for CLIA-approved laboratories. Although physicians, patients, and laboratory directors have lobbied for proficiency testing standards for those laboratories that provide high-complexity molecular and genetic testing services, CMS asserts that these issues lack sufficient urgency to warrant further regulation, and can be best considered in the context of existing CLIA regulations (Hudson 2006a). With at least a 33 percent failure rate of CLIA-certified laboratories that perform genetic testing to participate in

proficiency testing, and the inverse relationship between errors in diagnostic analyses and proficiency testing, the current regulatory position may be cause for concern (Hudson 2006b).

The FDA has not had a consistent position regarding jurisdiction over laboratory-developed genetic tests. It has authority to regulate them, but has exercised enforcement discretion. In September 2006, the FDA issued a draft guidance extending regulatory enforcement authority to a subset of home brew molecular tests known as *in vitro* diagnostic multivariate index assays (IVDMIAs) (FDA 2006). MIAs measure multiple analytes in the context of other clinical information and analyze the data with algorithms or software programs. The FDA targeted IVDMIAs for regulation because the algorithms often are proprietary, resulting in a physician's inability to interpret the results. It is anticipated that most IVDMIAs will require some level of FDA review before marketplace entry. It is noteworthy, however, that the FDA has not developed an overarching position regarding oversight of home brew assays as a class.

POOLING RESOURCES

Discovery, validation, qualification, and implementation of molecular biomarkers for the management of diseases, including cancer, will require dramatic changes in the practice of science, pharmaceutical and biotechnology development, and federal regulation, and will necessitate cooperation and collaboration on an unprecedented scale. These changes represent a cultural realignment in a direction that has, until recently, been anathema to constituents. To maximize productivity in the field of molecular biomarkers for cancer — the dividends of which accrue directly to improved disease-free survival for patients — large comprehensive and relational databases will be required. These should involve tens of thousands of patients followed longitudinally so that individual molecular (e.g., genomic, proteomic, metabolic) profiles can be compared to clinical characteristics of patients and pathologic characteristics of tumors (Dalton 2006). In this model, standardized methods will be used for database construction, common statistical algorithms will be employed for analyses, universally accepted trial designs will be implemented, and standardized analytical platforms will be applied. To extend this model, these relational databases ultimately could be adapted for use by practicing community physicians to truly individualize patient therapy. An integrated grid of molecular biomarkers could be used to define disease prognosis, predict responsiveness to specific targeted therapies, and anticipate and avoid patient-specific adverse reactions (Dalton 2006).

Collaboration and cooperation between stakeholders involved in biomarker development, application, and regulation may be the most expeditious path toward the translation of laboratory discovery into patient management.

These considerations suggest that collaboration and cooperation between stakeholders involved in biomarker development, application, and regulation may be the most expeditious path toward the translation of laboratory discovery into patient management. The cultural hurdles to this level of cooperation are significant in the context of the historically oppositional missions of academia and industry. Moreover, patient confidentiality, privacy rights, and the fear of discriminatory misuse of molecular data are fixed obstacles to the full implementation of this model (Dalton 2006).

To that end, the Pharmaceutical Research and Manufacturers of America (PhRMA) and the FDA have taken the first bold step in implementing this broad collaboration by creating a consortium for the development of biomarkers (Wagner 2007). The PhRMA/FDA Biomarker Consortium, established under the Foundation for the National Institutes of Health, was launched in October 2006. Other stakeholders include the NIH, CMS, academic institutions, and representatives from the private sector, including pharmaceutical, biotechnology, and diagnostics companies. The consortium will be open to institutions in the public and private sectors and will manage biomarker programs to ensure scientific rigor, appropriate prioritization and funding, and compliance with relevant statutes. The consortium will centralize activi-

ties focused on identifying, validating, and qualifying biomarkers that will be integrated into the clinical application of both new and previously approved marketed drugs. This represents one confident — but necessary — step toward realizing the goal of biomarker-based prognostic and predictive individualized medicine through requisite cooperation and collaboration.

CONCLUSION

Molecular biomarkers offer a clear path from the current curative model of clinical care to the development and implementation of preemptive prognostic and predictive medicine. Their evolution into mainstream clinical practice, however, is predicated on the development of strict paradigms centered on analytic validation and clinical qualification. In that regard, biomarker development and clinical application should have a firmly established basis of preclinical and clinical evidence, reflecting solid clinical trial design, analytical methodologies, and statistical rigor. Moreover, there may be benefits in centralizing federal regulatory oversight of these activities, including approval, marketing, and quality control in application, in the FDA and/or CMS. The recent establishment of the Biomarker Consortium is a necessary first step to pooling resources, standardizing approaches, and instituting collaborations across public and private sectors. This enterprise will facilitate the discovery and application of biomarkers that will support the development of new molecularly targeted therapeutics to achieve a truly individualized approach to patient care. BH

REFERENCES

Dalton WS, Friend SH. Cancer biomarkers: an invitation to the table. *Science*. 2006:312:1165–1168.

Faderl S, Hochhaus A, Hughes T. Monitoring of minimal residual disease in chronic myeloid leukemia. *Hematol Oncol Clin North Am*. 2004;18: 657–670, ix–x.

FDA (U.S. Food and Drug Administration). Center for Devices and Radiological Health, Office of In Vitro Diagnostic Device Evaluation and Safety. Draft guidance for industry, clinical laboratories, and FDA staff: in vitro diagnostic multivariate index assays. 2006. Available at: «http://www.fda.gov/cdrh/oivd/guidance/1610.pdf». Accessed Dec. 5, 2006.

Glas AM, Kersten MJ, Delahaye LJ, et al. Gene expression profiling in follicular lymphoma to access clinical aggressiveness and to guide the choice of treatment. *Blood*. 2005;105: 301–307.

Honjo S, Doran HE, Stiller CA, et al. Neuroblastoma trends in Osaka, Japan, and Great Britain 1970–1994, in relation to screening. *Int J Cancer*. 2003; 103:538–543.

Hudson KL. Genetic testing oversight. *Science*. 2006a;313:1853.

Hudson KL, Murphy JA, Kaufman DJ, et al. Oversight of US genetic testing laboratories. *Nat Biotechnol*. 2006b;24: 1083–1090.

Imperiale TF, Ransohoff DF, Itzkowitz SH, et al. Fecal DNA versus fecal occult blood for colorectal cancer screening in an average-risk population. *N Engl J Med*. 2004;351:2704–2714.

Iyer L, King CD, Whitington PF, et al. Genetic predisposition to the metabolism of irinotecan (CPT-11). Role of uridine diphosphate glucuronosyltransferase isoform 1A1 in the glucuronidation of its active metabolite (SN-38) in human liver microsomes. *J Clin Invest*. 1998;101:847–854.

Licking EF, Longman R. The new diagnostics companies. *Start-Up: Windhover's Review of Emerging Medical Ventures*. 2006;March:12–19.

Marshall E. Preventing toxicity with a gene test. *Science*. 2003;302:588–590.

Matloff ET, Brierley KL, Chimera CM. A clinician's guide to hereditary colon cancer. *Cancer J*. 2004;10:280–287.

Meijers-Heijboer H, van Geel B, van Putten WL, et al. Breast cancer after prophylactic bilateral mastectomy in women with a BRCA1 or BRCA2 mutation. *N Engl J Med*. 2001;345:159–164.

Miller JH, Kramer BS. Proteomics: promise and problems. *The Scientist*. 2006; 20:34.

Paik S, Shak S, Tang G, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med*. 2004;351: 2817–2826.

Petricoin EF, Ardekani AM, Hitt BA, et al. Use of proteomics patterns in serum to identify ovarian cancer. *Lancet*. 2002;359:572–577.

Pollack A. A crystal ball submerged in a test tube; genetic technology reshapes the diagnostics business. *New York Times*. 2006 Apr 13:C1.

Sidransky D. Emerging molecular markers of cancer. *Nat Rev Cancer*. 2002;2: 210–219.

Sjoblom T, Jones S, Wood LD, et al. The consensus coding sequences of human breast and colorectal cancers. *Science*. 2006;314:268–274.

Wagner JA, Williams SA, Webster CJ. Biomarkers and surrogate end points for fit-for-purpose development and regulatory evaluation of new drugs. *Clin Pharmacol Ther*. 2007;81:104–107.

Williams SA, Slavin DE, Wagner JA, et al. A cost-effectiveness approach to the qualification and acceptance of biomarkers. *Nat Rev Drug Discov*. 2006;5:897–902.

Wilson JF. The rocky road to useful cancer biomarkers. *Ann Intern Med*. 2006; 144:945–948.

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