criteria are not being met (*34*). It is unrealistic to think that the specialist genetics services will expand to cope with this, so the burden will fall on primary care and on hospital surgical clinics. It is here that information and education must be targeted. Explicit guidelines have been published for the follow-up care of individuals found to have predisposing mutations for breast, ovarian, and colorectal cancer (*20*, *35*). A widely available consensus statement with similarly explicit guidelines for family history criteria that may merit specialist referral for genetic testing might also be helpful (at present, it seems the best-publicized criteria are those put forward by commercial laboratories). Such guidelines would provide reassurance to clinicians beset by demand and uncertain how to respond; and they will also encourage providers of health care that they will not be asked to meet an open-ended commitment.

REFERENCES AND NOTES

1. C. J. M. Lips *et al.*, *N. Engl. J. Med.* **331**, 828 (1994); H. Z. Nourani, H. N. Khan, B. L. Gallie, A. S. Detsky, *Am. J. Hum. Genet.* **59**, 301 (1996); S. M. Powell *et al.*, *N. Engl. J. Med.* **329**, 1982 (1993).
2. B. Healey, *N. Engl. J. Med.* **336**, 1448 (1997).
3. R. Hubbard and R. C. Lewontin, *ibid.* **334**, 1192 (1996).
4. P. Kahn, *Science* **274**, 496 (1996).
5. N. J. Nelson, *J. Natl. Cancer Inst.* **88**, 70 (1996).
6. A. Marshall, *Nature Biotechnol.* **14**, 1642 (1996).
7. D. Smith, *Nature Med.* **3**, 709 (1997).
8. D. F. Easton *et al.*, *Am. J. Hum. Genet.* **56**, 265 (1995).
9. M. P. M. Richards *et al.*, *J. Genet. Counseling* **4**, 219 (1995); C. Lerman *et al.*, *J. Am. Med. Assoc.* **275**, 1885 (1996).
10. P. Williamson *et al.*, *J. R. Coll. Physicians London* **30**, 443 (1996).
11. H. J. Jarvinen, J. P. Mecklin, P. Sistonen, *Gastroenterology* **108**, 1405 (1995).
12. M. Dunlop and H. Campbell, *Br. Med. J.* **314**, 1779 (1997).
13. J. Streuwing *et al.*, *N. Engl. J. Med.* **336**, 1401 (1997).
14. S. Laken *et al.*, *Nature Genet.* **17**, 79 (1997).
15. Oncormed Inc., BRCA1/BRCA2 Fact Sheet (1997).
16. M. Krainer *et al.*, *N. Engl. J. Med.* **336**, 1416, 1997; F. J. Couch *et al.*, *ibid.*, p. 1409.
17. J. Green, M. P. M. Richards, H. Statham, *J. Genet. Counseling* **6**, 45 (1997).
18. F. S. Collins, *N. Engl. J. Med.* **334**, 186 (1996).
19. S. Mazoyer, A. M. Dunning, O. Serova, *Nature Genet.* **14**, 253 (1996).
20. W. Burke *et al.*, *J. Am. Med. Assoc.* **277**, 997 (1997).
21. D. Easton, *Nature Genet.* **15**, 210 (1997).
22. N. Hallowell *et al.*, *Psychol. Health Med.* **2**, 149 (1997).
23. M. P. M. Richards, *Public Understanding Sci.* **5**, 217 (1996).
24. D. Schrag, K. M. Kuntz, J. E. Garber, J. C. Weeks, *N. Engl. J. Med.* **336**, 1465 (1997).
25. K. Rothenberg *et al.*, *Science* **275**, 1755 (1997).
26. K. L. Hudson, K. H. Rothenberg, L. B. Andrews, M. J. Ellis Kahn, F. S. Collins, *ibid.* **270**, 391 (1995).
27. Association of British Insurers, Information Sheet: Life Insurance and Genetics (ABI, London, 1997).
28. NAPBC and NIH-DOE ELSI Working Group, "Recommendations on genetic information and the workplace" (NAPBC, U.S. Public Health Service Office on Women's Health, Washington, DC, 1997). For further information see www.napbc.org.
29. M. L. Brown and L. G. Kessler, *J. Natl. Cancer Inst.* **87**, 1131 (1995).
30. Task Force on Genetic Testing of the NIH-DOE Working Group on Ethical, Legal and Social Implications of Human Genome Research: Promoting Safe and Effective Genetic Testing in the United States.
31. Nuffield Council on Bioethics, *Genetic Screening: Ethical Issues* (Nuffield Foundation, London, 1993).
32. American Society of Clinical Oncology, *J. Clin. Oncol.* **14**, 1730 (1996).
33. American Society of Human Genetics, *Am. J. Hum. Genet.* **55**, i (1994).
34. F. M. Giardiello *et al.*, *N. Engl. J. Med.* **336**, 823 (1997).
35. W. Burke *et al.*, *J. Am. Med. Assoc.* **277**, 915 (1997).

36. I thank M. Richards for helpful discussions and M. Bobrow, L. Brody, F. Collins, D. Easton, M. Ponder, and M. Richards for critical comments on the manuscript. B.P. is a Gibb Fellow of the Cancer Research Campaign (CRC).

Nucleic Acid–Based Methods for the Detection of Cancer

David Sidransky

Continued elucidation of the genetic changes that drive cancer progression is yielding new and potentially powerful nucleic acid–based markers of neoplastic disease. Pilot studies indicate that these markers can be used to detect cancer cells in a variety of clinical settings with unprecedented precision. Nucleic acid–based markers may prove to be valuable tools for early detection of cancer in asymptomatic individuals, for confirmation or exclusion of a cancer diagnosis that is based on suspicious but nondiagnostic clinical material, for assessment of tumor burden in cancer patients, and for assessment of response to preventive approaches applied to healthy individuals who are at high risk of developing cancer. Examples of these markers, their potential applications, and the current practical limitations on their clinical use are reviewed here.

Recent discoveries in genetics and molecular biology have revolutionized our understanding of cancer initiation and progression. We now know that cancer is a heterogeneous group of diseases, each composed of a complex array of genetic changes driving uncontrolled growth and metastatic spread. Although this understanding has stimulated the development of innovative molecular therapies for cancer, successful introduction of these therapies into the clinical setting has been rare. Thus, a simple molecular cure for the most common cancers must still be viewed as a long-term goal. However, the war on cancer has many fronts. Identification of the genetic changes that drive cancer progression is also providing us with a variety of molecular markers and tests that may ultimately redefine the criteria for cancer diagnosis and provide new avenues for early detection. Long before molecular cures for cancer arrive, accurate molecular diagnosis may change our clinical approach to and management of cancer patients. Here I will review the status of promising molecular tests for cancer, focusing primarily on nucleic acid–based diagnosis of epithelial cell malignancies, which account for the overwhelming number of cancer deaths worldwide.

The author is at The Johns Hopkins University School of Medicine, Department of Otolaryngology—Head and Neck Surgery, Division of Head and Neck Cancer Research, 818 Ross Research Building, 720 Rutland Avenue, Baltimore, MD 21205–2195, USA. E-mail: dsidrans@welchlink.welch.jhu.edu

Types of Molecular Markers

Strong evidence supports the concept that cancer is a genetic disease that involves clonal evolution of transformed cells (*1*). Cancer cells arise through the accumulation of mutations, either inherited (germline) or acquired (somatic), in critical proto-oncogenes and tumor suppressor genes. Each mutation may provide an additional growth advantage to the transformed cells as they dominate their normal counterparts (*2*, *3*). The genetic alterations that arise during tumorigenesis can be used as targets for detection of cancer cells in clinical samples. DNA is an ideal substrate for molecular diagnosis because it readily survives the adverse conditions experienced by many clinical specimens and it can be rapidly amplified by polymerase chain reaction (PCR)–based techniques, thus diminishing the amount of starting material needed.

In addition to specific mutations in oncogenes and tumor suppressor genes, changes in DNA repeat sequences, called microsatellites (*4*), can also be used as markers to detect the clonal evolution of neoplastic cells. Because they are highly polymorphic, microsatellite markers allow distinction of maternal and paternal alleles. Typically, paired samples of normal DNA (usually from blood lymphocytes) and DNA from a

clinical sample (a tumor or bodily fluid such as urine) are compared. The loss of one allele in the clinical sample [loss of heterozygosity (LOH)] results from chromosomal deletion or mitotic combination and is commonly thought to represent the second genetic inactivation step in the complete loss of a tumor suppressor gene locus (*5*).

Microsatellite analysis can also detect the presence of a new allele (detected as a mobility shift on electrophoretic gels) indicative of microsatellite instability. Widespread microsatellite instability, manifested as expansion or deletion of many repeat elements in tumor DNA, is particularly common in colorectal tumors; and in patients with hereditary nonpolyposis colorectal carcinoma (HNPCC), it is caused by mutations of DNA mismatch repair genes (*6*). Microsatellite instability can occur in many tumor types and can inactivate tumor suppressor genes, but more often it occurs in anonymous stretches of noncoding DNA. The detection of either of these genetic changes in a clinical sample (LOH or instability, or both) demonstrates the presence of a clonal population of cells that share altered genetic information, which is a characteristic of cancer cells.

Mutations in proto-oncogenes and tumor suppressor genes can produce vast changes in the expression of many other genes. These changes can be assessed at the RNA level, although RNA is a less suitable substrate for clinical diagnosis than DNA because it is readily degraded. However, careful isolation of RNA from clinical samples with subsequent conversion to cDNA and amplification [called reverse transcriptase (RT)–PCR] may be a more viable approach to evaluating gene expression in the blood, lymph nodes, and bone marrow of cancer patients. Moreover, normal and neoplastic cells are distinguished by the differential expression of hundreds of cellular genes. New RNA-based methods for gene discovery that can track these changes in expression, including cDNA chip arrays (*7*) and serial analysis of gene expression (SAGE) (*8*), will produce an ever-growing number of potential tumor markers.

Another new marker is telomerase (discussed below), a ribonucleoprotein enzyme that extends the sequences at chromosomal ends (telomeres) and is active in >90% of primary human tumors and cell lines (*9*) but inactive in most normal cells (*10*).

Sensitivity and Specificity

Looking for cancer cells in a clinical sample that contains a predominance of normal cells can be like looking for the proverbial needle in a haystack. This is especially true for molecular tests, which, unlike cytological tests based on morphological assessment of individual or clustered cells, usually begin with the preparation of a specific substrate such as DNA from the admixture. The ratio of tumor cells to normal cells varies considerably from one organ system to another and from one individual to another. Thus, molecular tests must be developed with a clear understanding of the clinical problem and the limits of the technology. A simple molecular test to identify bladder cancer cells in urine, where 50% or more of the DNA may be derived from sloughed-off tumor cells, may be wholly inadequate for identification of lung cancer cells in sputum, where ≤0.2% of the DNA is likely to be isolated from tumor cells. Conversely, exquisitely sensitive PCR-based approaches that can detect an abnormal transcript from one cancer cell among 10^6 normal cells may identify changes in single cells or cell clusters that are not yet clonal or will not definitively progress to cancer (*11*). Identification of molecular changes with this sensitivity may serve to identify patients at risk of developing cancer but may be unsuitable for early detection. The precise cutoff for accurate detection of clinical disease has not been determined. Only prospective testing in patients at risk of cancer will empirically identify the critical threshold for accurate detection of the smallest tumors.

Once the reliability of a technique is established through feasibility studies, its sensitivity and specificity must then be assessed in formal clinical trials. Sensitivity refers to how often the test identifies cancer when it is present, and specificity refers to how often the test correctly identifies cancer. If the prevalence of a specific cancer type is low in the general population, the test must be exquisitely specific; otherwise more patients without cancer may test positive.

Applications

Early detection. Because successful treatment of most cancers depends on early detection, there is a critical need for new early detection approaches. Based on our emerging knowledge of the underlying genetic events that lead to cancer initiation and progression, pilot studies have shown that oncogenes and tumor suppressor gene mutations can be successfully identified in bodily fluids that drain from the organ affected by the tumor (Table 1). Using sensitive assays, investigators have found *ras* or *p53* mutations in many bodily fluids of patients, including blood (*12–18*). In all of these studies, the identical mutation present in the primary tumor was identified in the bodily fluid tested from affected patients.

ras and *p53* mutations have been used as molecular markers in these studies because they commonly occur in the tumor types tested (*19*) and because they may provide information about the staging of the tumor; for example, in colon cancer, *ras* mutations are an early event in tumorigenesis, whereas *p53* mutations usually occur in invasive tumors (*2*). It would follow that APC mutations, which occur in more than 70% of colon adenomas (precursors to cancer), might also be valid markers (*20*). However, identification of all the possible mutations in the coding region of a gene, especially when it is the size of APC (8.5 kb) is daunting. Such "mutation scanning" to detect these alterations in an admixture of normal and neoplastic cells is not presently feasible. With the exception of *K-ras* mutations (clustered at codons 12 and 13) or a few mutation "hotspots" in *p53* (1.2 kb), this technological barrier is preventing the development of the necessary assays for initiation of clinical trials to validate this approach.

Because of these technical limitations, there is a great need to identify other clonal DNA-based markers. Microsatellite analysis is emerging as an important and relatively easy alternative for cancer detection. In contrast to the use of specific probes to identify oncogene mutations, these molecular alterations are easily identified with one set of primers for all samples. In a retrospective analysis of urine samples from 25 patients, microsatellite markers successfully detected over 90% of bladder tumors (*21*). In a follow-up study, 10 of 11 recurrences were detected prospectively and two patients had a positive test several months before the clinical cancer was visualized by bladder inspection (cystoscopy) (*22*). A multi-institutional trial to test for bladder cancer recurrence using a panel of 20 microsatellite markers is already under way.

As noted above, microsatellite alterations (low-level instability) can be also be found in tumors without mismatch repair deficits. PCR with subsequent electrophoretic separation of the PCR products can identify these shifts at a sensitivity of ~1 neoplastic cell among 500 normal cells, which appears to be sufficient for clinical detection in many situations. Certain microsatellite markers are particularly unstable in human tumors; these markers often contain larger repeats, particularly tetranucleotides (*23*). Interestingly, although some microsatellite markers are unstable in virtually all tumors, others are unstable only in specific tumor types. The mechanism underlying this phenomenon is unknown but may involve flanking DNA sequences,

tissue-specific expression of genes in the surrounding chromosomal region, or an underlying DNA repair deficit.

As mentioned above, the ribonucleoprotein enzyme telomerase is expressed selectively in virtually all primary tumors and therefore has emerged as a promising molecular marker for cancer detection. Currently, telomerase activity in clinical samples is measured by the TRAP (telomerase repeat amplification protocol) assay, which requires protein extraction and subsequent primer-directed PCR amplification of telomere extensions (*24*). The specificity of this approach has been lower than that reported in studies using detection of DNA alterations (*25–27*) (Table 1). The recent cloning of the human telomerase catalytic component (*28*) may allow development of improved assays.

Tumor burden. Molecular markers can also be used to assess the migration of tumor cells locally or into the bloodstream. Surgery remains the most effective treatment for most localized primary tumors, but tumor cells often spread beyond the surgical margins and may evade detection by standard light microscopy. Because the tumor cells are vastly outnumbered by normal cells in this situation, very sensitive detection techniques are required. In one study of patients, specific *p53* mutations were used to identify infiltrating tumor cells in surgical margins beyond the resection border (*29*). In ~50% of the patients, tumor cells harboring the same mutations identified in the primary tumor were detected in apparently "clean" margins or lymph nodes. Despite radiation treatment, about one-third of the patients with these mutations went on to recur, often developing new tumors adjacent to or within the area identified as positive by molecular analysis. A similar analysis of *p53* and *ras* mutations has identified tumor cells in apparently disease-free lymph nodes of colorectal and lung cancer patients, but the clinical outcome of positive patients was not provided for critical appraisal (*30*). The determination of node status is critical for precise staging of tumors and for treatment decisions.

In addition to local spread, malignant cells can metastasize; that is, enter the bloodstream, disseminate, and grow in other organs. In light of earlier studies indicating that cancer patients have large amounts of circulating DNA in serum or plasma (*31*), blood samples are now being analyzed for nucleic acid markers such as *K-ras* mutations and microsatellite alterations (*32, 33*). In 29% of 21 patients with head and neck squamous cell carcinoma (HNSCC) cancer and in 71% of 21 patients with small-cell lung cancer (SCLC), LOH or microsatellite alterations were detected in serum or plasma (*33*). In the HNSCC study, the positive patients had larger tumors and a poorer prognosis. The higher incidence of plasma DNA alterations in SCLC patients may reflect the tendency of these tumors to metastasize early (*33*). Although analysis of serum nucleic acid markers does not currently allow early detection of tumors, it may provide useful information on tumor burden and response to therapy.

Analysis of whole blood and bone marrow (BM) for abnormal transcripts derived from neoplastic cells is routinely used to monitor patients with chronic myelogenous leukemia. More recently, transcripts expressed exclusively or preferentially in cancer cells have been targets of RT-PCR–based detection strategies in patients with solid tumors. For example, tyrosine hydroxylase transcripts were found to correlate with micrometastatic BM disease in neuroblastoma, and tyrosine transcript levels in melanoma may predict a poor prognosis (*34*). RT-PCR approaches targeting cytokeratins, adhesion molecules, tyrosine kinases, and prostate-specific markers [including prostate-specific antigen (PSA) and prostate-specific membrane antigen PSM] to detect micrometastatic disease have been tested in various tumor types, including primary breast, gastric, colorectal, lung, and prostate cancer (*35*). However, issues of specificity remain because of illegitimate expression of these markers in normal cells and down-regulation of the markers in tumor cells. One recent study suggests that RT-PCR of PSA in bone marrow shows high specificity (no false positives in 53 control patients) for micrometastatic disease in prostate cancer (*36*). Testing BM may be more relevant in some cases, because animal studies suggest that only a small portion of metastatic cells actually settle and develop into metastatic deposits in various organs.

Adjuncts to cytology and histopathology. Needle aspirates from various organs are often used to establish the cancer diagnosis when there is a suspicious mass. In some cases, it is difficult to distinguish between benign or preneoplastic lesions and frank cancer. Recently, telomerase was detected in all 11 follicular carcinomas of the thyroid but in only 8 of 33 benign follicular tumors and never in normal thyroid tissue (*37*).

Table 1. Selected feasibility trials employing molecular diagnosis of clinical samples. This table lists selected pilot or feasibility trials using accessible clinical bodily samples in molecular detection approaches. These studies have employed both retrospective and prospective collection of samples, but only the study by Steiner *et al.* (*22*) reports prospective follow-up of the patient cohort. Studies using gene targets (*ras* and *p53*) report sensitivity of the molecular assays as a fraction of patients with tumors containing the sought-after mutation. The other studies report sensitivity as a fraction of all patients with cancer, regardless of the molecular status of their tumor. When microsatellites are used as targets, the number of markers in each study varies and is listed in parentheses. The upper limit of detection denotes the upper limit of sensitivity for the assay as a dilution of cancer cells among normal cells. Nipple aspirates and ejaculates are potential but unproven clinical samples for the detection of breast and prostate cancer, respectively. Sensitivity and specificity for detecting cancer are listed as reported in each study.

Cancer type	Clinical sample	Genetic marker	Upper limit of detection	Patients (*n*)	Controls (*n*)	Sensitivity (%)	Specificity (%)	Reference
Head and neck	Saliva	*p53*	1/10,000	7	0	71	100	*49*
		Telomerase	1/10,000	44	22	32	95	*25*
Lung	Sputum	*ras/p53*	1/10,000	10	5	80	100	*17*
		ras	1,10,000	5	30	100	100	*16*
		Microsatellites (*4*)	1/500	5	0	60	100	*50*
Colon	Stool	*ras*	1/10,000	9	6	88	100	*13*
		Telomerase	1/10,000	15	9	60	100	*26*
Pancreas	Stool	*ras*	1/10,000	11	3	66	100	*15*
	Juice	*ras*	1/100,000	7	3	100	100	*14*
Bladder	Urine	*p53*	1/10,000	3	3	100	100	*12*
		Microsatellites (*13*)	1/500	20	5	95	100	*21*
		Microsatellites (*20*)	1/500	21	0	91	100	*22*
		Telomerase	1/10,000	26	83	62	96	*27*

Thus, detection of telomerase in needle biopsies from suspicious thyroid nodules may help establish the diagnosis of follicular carcinoma before proceeding to thyroidectomy. Others have used telomerase to correctly establish the diagnosis from three suspicious but not diagnostic needle biopsies taken from breast cancers (*38*).

The Pap smear of the cervix is the single most successful effort in screening for cancer that has been made in this century. Virtually all cervical cancers are associated with human papilloma virus (HPV) infection, and investigators have developed a sensitive molecular test to identify HPV sequences in liquid cytology medium. In older women (who have a low prevalence of HPV infection) with borderline abnormalities, HPV testing identifies ~90% of individuals who have underlying high-grade neoplasia (*39*).

Another perplexing problem in pathology is the need to identify the primary tumor when a patient presents only with a metastatic lymph node. This is especially common in the neck, where an occult primary HNSCC tumor may be difficult to identify. Microsatellite analysis has proven useful even when random biopsies of the oral cavity and hypopharynx do not reveal the primary malignant focus (*40*). In preliminary studies with HNSCC, it was found that in 6 of 10 patients, the same genetic changes identified in the metastatic deposits were present in at least one random mucosal biopsy (*40*). In two cases, the primary tumor subsequently recurred in the predicted anatomical site identified by molecular analysis.

Intermediate biomarkers. Clonal genetic changes that can reliably predict the occurrence of neoplasia well in advance of clinical cancer may have a role as intermediate biomarkers in cancer prevention studies. New opportunities may thus exist to test chemopreventive agents in populations without waiting many years for accurate statistical analysis from commonly used endpoints, such as survival or the onset of cancer.

The notion that oncogene or tumor suppressor gene mutations can be used as markers for early preneoplastic disease is supported by the recent observation of clonal *K-ras* mutations in hyperplastic intestinal crypts (*41*). Until the emergence of an *APC* mutation in these cell populations leads to dysplasia, progression to cancer is unlikely (*41*). Other studies have demonstrated the presence of *K-ras* and *APC* mutations as well as microsatellite instability in the preneoplastic mucosa of patients with ulcerative colitis, who are also at high risk for colon cancer (*42*). *K-ras* mutations have also been found in the sputum of smokers without lung cancer (*43*). Thus, the identification of *K-ras* mutations, especially in low proportion to normal DNA (for example, 1 in 10,000 to 100,000), may signal the presence of preneoplastic clones in addition to overt clinical lesions and supports the role of *K-ras* as an intermediate marker for monitoring and chemopreventive studies.

p53 mutations are induced by a variety of endogenous and exogenous compounds. In patients with skin cancer, pyrimidine dimer mutations occur at specific sites in *p53* as a result of ultraviolet exposure. These mutations can also be found in normal skin of sun-exposed individuals, and their frequency correlates with overall sun exposure (*44*). Recently, a sensitive assay to detect these changes was used to compare the efficacy of various sunscreens in a mouse model (*45*). *p53* mutation hotspots can be found in other tumor types (for example, codon 249 mutations in liver cancer) and are much easier to test for than a whole array of mutations.

From the Bench to the Bedside

Figure 1 summarizes some of the clinical applications of the nucleic acid markers. Continued development of these markers will require the establishment of large biorepositories containing paired clinical samples of blood, tumors, and bodily fluids. To bring these new molecular approaches to the clinic, it is essential to carry out large well-controlled trials. Newly defined high-risk populations, such as carriers of germline mutations in cancer genes, will be crucial for successful implementation of these trials. These new molecular approaches can be tested quickly in populations at high risk for disease. The information garnered from these trials can then be incorporated into routine monitoring and perhaps screening

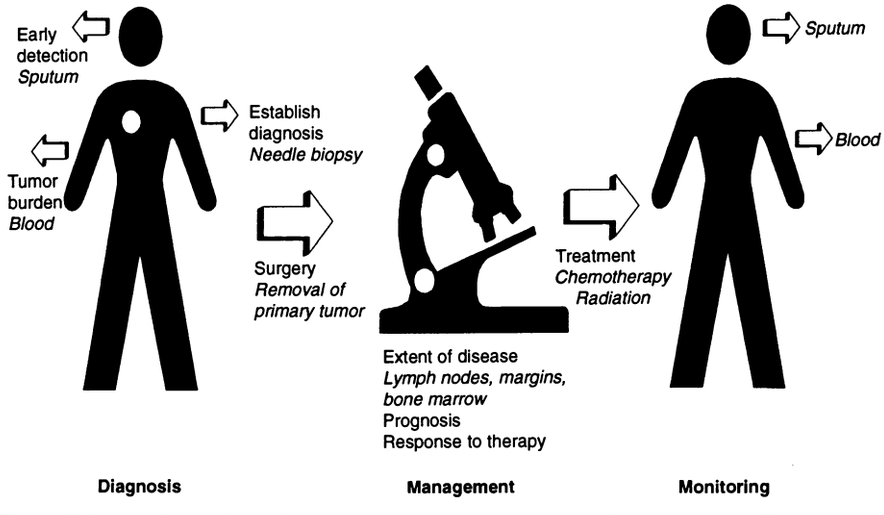

Fig. 1. Molecular diagnostic applications in the overall management of cancer patients. The schematic depicts the opportunities for molecular analysis of clinical samples in a lung cancer patient as the patient moves from diagnosis, to surgery (for a localized tumor), to additional treatment, and eventually to long-term monitoring. These applications (with the exception of prognosis) are discussed in the text.

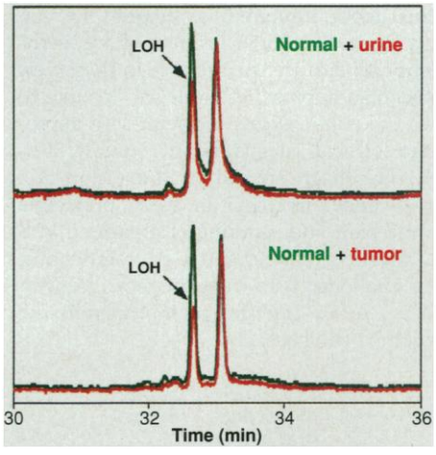

Fig. 2. Molecular detection of bladder cancer by fluorescence-based PCR, microcapillary electrophoresis, and laser detection. The figure shows electropherograms of PCR products derived from PCR amplifications of the interferon (IFNA) microsatellite locus (chromosomal arm 9p21) in various samples from the same patient (provided by R. Mathie, University of California, Berkeley). Products from the normal sample (lymphocytes) were generated with energy transfer (ET) primers and detected in the green channel (537 to 567 nm), and those from tumor and urine samples were generated with ET primers detected in the red channel (>590 nm). The reduced intensity of one allelic band in the urine and tumor samples as compared to the corresponding normal DNA reflects LOH. The recent development of 96-well capillary array devices supports this assay as a high-throughput approach for cancer detection (*47*).

for these patients and the population at large. As these patients are recruited for such trials, important ethical issues, including informed consent and insurance coverage, must be appropriately addressed.

Despite the great promise of these new molecular approaches for cancer detection, much of the current technology limits their implementation into routine clinical use even for high-risk populations. High-throughput technologies have to be developed and integrated to make these assays a reality (Fig. 2). Genosensor arrays and microcapillary systems may make these tests accessible in the near future (*46*, *47*).

A positive molecular test is only useful if the tumor can be localized and eradicated. Current imaging approaches cannot reliably detect small tumor masses. For many patients, identification of the primary tumor will result in cure but for others, a positive molecular test may be followed by negative imaging studies. Continued improvements in magnetic resonance metabolic imaging and fluorescence imaging technologies will likely improve the ability of clinicians to localize small, perhaps even microscopic, lesions (*48*).

In our present health care environment, it is difficult to initiate new tests without extensive cost-benefit analysis and concerns about insurance coverage. One can only hope that large insurers and government agencies see the promise in these new molecular approaches and are willing to give the public access to them, with appropriate ethical safeguards, in a timely fashion. The diagnosis and rapid excision of a small cancerous lesion are vastly preferable to the pain and suffering of a patient with an advanced cancer and a poor prognosis. Our challenge is to translate new discoveries in cancer genetics promptly from the bench to the bedside.

REFERENCES AND NOTES

1. P. J. Fialkow, *Biochim. Biophys. Acta* **458**, 283 (1976); P. C. Nowell, *Science* **194**, 23 (1976).
2. E. R. Fearon and B. Vogelstein, *Cell* **61**, 709 (1990).
3. D. Sidransky, T. Mikkelsan, W. Cavenee, B. Vogelstein, *Nature* **355**, 841 (1992).
4. J. L. Weber and P.E. May, *Am. J. Hum. Genet.* **44**, 388 (1989); J. Weissenbach *et al.*, *Nature* **359**, 794 (1992).
5. A. G. Knudson Jr., *Proc. Natl. Acad. Sci. U.S.A.* **68**, 820 (1971); *Cancer Res.* **45**, 1437 (1985).
6. K. W. Kinzler and B. Vogelstein, *Cell* **87**, 159 (1996); G. Marra and C. R. Boland, *Gastroenterol. Clin. North Am.* **25**, 755 (1996).
7. J. DeRisi *et al.*, *Nature Genet.* **14**, 457 (1996); M. Schena *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 10614 (1996); M. Schena, D. Shalon, R. W. Davis, P. O. Brown, *Science* **270**, 467 (1995).
8. L. Zhang *et al. Science* **276**, 1268 (1997).
9. E. Blackburn, *Nature* **350**, 569 (1991); N. W. Kim *et al.*, *Science* **266**, 2011 (1994).
10. O. Yamada, T. Motoji, H. Mizoguchi, *Biochim. Biophys. Acta.* **1314**, 260 (1996); N. P. Weng, B. L. Levine, C. H. June, R. J. Hodes, *J. Exp. Med.* **183**, 2471 (1996); S. Yasumoto *et al.*, *Oncogene* **13**, 433 (1996).
11. F. Aguilar, C. C. Harris, T. Sun, M. Hollstein, P. Cerutti, *Science* **264**, 1317 (1994).
12. D. Sidransky *et al.*, *ibid.* **252**, 706 (1991).
13. D. Sidransky *et al.*, *ibid.* **256**, 102 (1992).
14. M. Tada *et al.*, *Cancer Res.* **53**, 2472 (1993).
15. C. Caldas *et al.*, *ibid.* **54**, 3568 (1994).
16. N. E. Mills *et al.*, *J. Natl. Cancer Inst.* **87**, 1056 (1995).
17. L. Mao, R. H. Hruban, J. O . Boyle, M. Tockman, D. Sidransky, *Cancer Res.* **54**, 1634 (1994).
18. M. Tobi, F. C. Luo, Z. Ronai, *J. Natl. Cancer Inst.* **86**, 1007 (1994).
19. J. L. Bos *et al.*, *Nature* **327**, 293 (1987); M. Hollstein, D. Sidransky, B. Vogelstein, C. C. Harris, *Science* **253**, 49 (1991).
20. S. M. Powell *et al.*, *N. Engl. J. Med.* **329**, 293 (1987).
21. L. Mao *et al.*, *Science* **271**, 659 (1996).
22. G. Steiner, M. P. Schoenberg, J. F. Linn, L. Mao, D. Sidransky, *Nature Med.* **3**, 621 (1997).
23. L. Mao *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 9871 (1994).
24. W. E. Wright, J. W. Shay, M. A. Piatyszek, *Nucleic Acids Res.* **23**, 3794 (1995).
25. J. Califano *et al.*, *Cancer Res.* **56**, 5720 (1996).
26. K. Yoshida *et al.*, *Br. J. Cancer* **75**, 548 (1997).
27. K. Yoshida *et al.*, *Cancer* **79**, 362 (1997).
28. T. M. Nakamura *et al.*, *Science* **277**, 955 (1997); M. Meyerson *et al.*, *Cell* **90**, 785 (1997).
29. J. A. Brennan *et al.*, *N. Engl. J. Med.* **332**, 429 (1995).
30. N. Hayashi *et al.*, *Cancer Res.* **54**, 3853 (1994); S. A. Ahrendt *et al.*, *Clin. Cancer Res.* **3**, 1207 (1997).
31. B. Shapiro *et al.*, *Cancer* **51**, 2116 (1983); S. A. Leon, *Cancer Res.* **37**, 646 (1977).
32. M. Stroun *et al.*, *Oncology* **46**, 318 (1989); M. Stroun, P. Anker, J. Lyautey, C. Lederrey, P. Maurice, *Eur. J. Clin. Oncol.* **23**, 707 (1987); V. Vasioukhin *et al.*, *Cancer Epidemiol. Biomark. Prev.* **3**, 67 (1994).
33. H. Nawroz, W. Koch, P. Anker, M. Stroun, D. Sidransky, *Nature Med.* **2**, 1035 (1996); X. Q. Chen *et al.*, *ibid.*, p. 1034.
34. B. Smith *et al.*, *Lancet* **338**, 1227 (1991); A. J. Foss *et al.*, *Br. J. Cancer* **72**, 155 (1995); P. Brossart *et al.*, *Cancer Res.* **55**, 4065 (1995).
35. Y. H. Datta, P. T. Adams, W. R. Drobyski, *J. Clin. Oncol.* **12**, 475 (1994); M. Gerhard *et al.*, *ibid.*, p. 725; M. Krismann *et al.*, *ibid.* **13**, 2769 (1995); A. E. Katz *et al.*, *Cancer* **75**, 1642 (1995).
36. A. Zippelius *et al.*, *J. Clin. Oncol.* **15**, 2701 (1997).
37. C. B. Umbricht *et al.*, *Cancer Res.* **57**, 2144 (1997).
38. T. Sugino *et al.*, *Int. J. Cancer* **69**, 301 (1996).
39. M. E. Sherman *et al.*, *Cancer* **81**, 89 (1997); M. Manos, personal communication.
40. J. Califano and D. Sidransky, unpublished data.
41. J. Jen *et al.*, *Cancer Res.* **54**, 5523 (1994).
42. M. S. Redston, N. Papadopoulos, C. Caldas, K. W. Kinzler, S. E. Kern, *Gastroenterology* **108**, 383 (1995); T. A. Brentnall *et al.*, *Cancer Res.* **56**, 1237 (1996).
43. T. Minamoto *et al.*, *Cancer* **75** (suppl. 6), 1520 (1995); Z. Ronai, M. S. Yabubovskaya, E. Zhang, G. A. Belitsky, *J. Cell. Biochem. Suppl.* **25**, 172 (1996).
44. H. Nakazawa *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 360 (1994).
45. H. N. Ananthaswamy *et al.*, *Nature Med.* **3**, 510 (1997).
46. J. G. Hacia, L. C. Brody, M. S. Chee, S. P. Fodor, F. S. Collins, *Nature Genet.* **14**, 441 (1996); W. W. Gibbs, *Sci. Am.* **275**, 42 (1996); J. Ju, A. N. Glazer, R. A. Mathies, *Nature Med.* **2**, 246 (1996).
47. Y. Wang *et al.*, *J. Electrophoresis* **18**, 1742 (1997).
48. S. P. Schantz and R. R. Altano, *J. Cell Biochem.* **17F**, 199 (1993); H. Degani, V. Gusis, D. Weinstein, S. Fields, S. Strano, *Nature Med.* **3**, 780 (1997); H. N. Wagner Jr., *Semin. Nucl. Med.* **26**, 194 (1996).
49. J. B. Boyle *et al.*, *Am. J. Surgery* **168**, 429 (1994).
50. M. Miozzo *et al.*, *Cancer Res.* **56**, 2285 (1996).

51. Supported in part by National Cancer Institute grant CA-88184-01 and by Oncor, Inc. Under an agreement between Oncor and the Johns Hopkins University, D.S. is entitled to a share of sales royalties received from Oncor by the university. D.S. is also a member of the Scientific Advisory Board of OncorMed, Inc., an Oncor subsidiary, which is commercializing some of Oncor's technology.