Early Detection of Cancer: Past, Present, and Future

Joshua D. Schiffman, MD, Paul G. Fisher, MD, and Peter Gibbs, MBBS, FRACP, MD

OVERVIEW

Screening in both healthy and high-risk populations offers the opportunity to detect cancer early and with an increased opportunity for treatment and curative intent. Currently, a defined role for screening exists in some cancer types, but each screening test has limitations, and improved screening methods are urgently needed. Unfortunately, many cancers still lack effective screening recommendations, or in some cases, the benefits from screening are marginal when weighed against the potential for harm. Here we review the current status of cancer screening: we examine the role of traditional tumor biomarkers, describe recommended imaging for early tumor surveillance, and explore the potential of promising novel cancer markers such as circulating tumor cells (CTC) and circulating tumor DNA. Consistent challenges for all of these screening tests include limited sensitivity and specificity. The risk for overdiagnosis remains a particular concern in screening, whereby lesions of no clinical consequence may be detected and thus create difficult management decisions for the clinician and patient. If treatment is pursued following overdiagnosis, patients may be exposed to morbidity from a treatment that may not provide any true benefit. The cost-effectiveness of screening tests also needs to be an ongoing focus. The improvement of genomic and surveillance technologies, which leads to more precise imaging and the ability to characterize blood-based tumor markers of greater specificity, offers opportunities for major progress in cancer screening.

INTRODUCTION TO CANCER SCREENING AND TUMOR MARKERS FOR EARLY CANCER DETECTION

Tumor markers have been used for decades in oncology. Tumor markers are biomarkers found in blood, urine, cerebrospinal fluid, or other body tissues that are elevated in association with cancer. Tumor markers can, in theory, be used for screening, diagnosis, staging, or disease monitoring. However, to date, many tumor markers have demonstrated poor accuracy and efficacy, particularly among the most prevalent cancers. To understand biomarkers and other tests employed for earlier detection of new or recurrent cancer, one needs to understand a number of epidemiologic concepts. Simply defined, screening is the use of a test among individuals with a population risk for or higher probability of cancer in order to detect cancer sooner (secondary prevention) or prevent its complications (tertiary prevention). Rarely, a screening test is used to prevent cancer (primary prevention), such as the Papanicolaou (Pap) test to find precancerous cellular changes in the cervix. When screening is used to monitor for cancer recurrence, the term surveillance is commonly used instead.

For screening to be efficacious, a number of conditions are necessary. The cancer should be an important cause of morbidity and mortality. A proven, safe, and acceptable test should exist to detect early-stage disease. The natural history of the cancer should be understood. The cancer should have a recognizable latent or early asymptomatic stage. In the absence of intervention, all or most cases in a preclinical phase should progress to a clinical phase. Pseudocancer, or even overdiagnosis of a benign cancer that would never progress, can be problematic in this situation. Safe and effective treatment must be available. Finally, the screening test should be
Among patients with undiagnosed cancer, a number of screening biases must always be remembered. Volunteers for screening are often healthier. Screening is susceptible to lead-time bias, that is, simply advancing the time of cancer diagnosis without changing the ultimate outcome. Length bias allows screening to detect more protracted, slower cancers, than rapid, severe forms, which leads to better outcomes in screened-detected cases because cancers with a better prognosis are being detected through the very screening modality being studied. Finally, screening can overdiagnose, which means it can detect premalignant lesions or early invasive cancers that would have never required any medical intervention in the lifetime of the patient. The latter has resulted in an epidemic of prostate cancer and ductal carcinoma in situ, many of which may never have been symptomatic or lethal.

When evaluating the utility of screening, one must always consider the test’s accuracy (or relative lack of error). Sensitivity indicates the ability of the test to identify correctly those who have cancer among the population with cancer (true-positives/[true-positives + false-negatives]), whereas specificity indicates the ability of the test to identify correctly those who do not have cancer among the population without cancer (true-negatives/[true-negatives + false positives]). No screening test can have 100% sensitivity and 100% specificity, and in general for many cancer screens, sensitivity hovers in the 70% to 80% range with specificity slightly lower at approximately 60% to 70%.

Perhaps most vexing about screening for cancer is the paradox of cancer epidemiology: cancer in the aggregate over a lifetime is common, while at any one time one specific cancer is rare. That is, at any given time, cancer prevalence by specific type is low, and a single asymptomatic individual has a low risk of cancer. This is crucial to understanding the limitations of screening tests, since the positive predictive value (PPV) of a test (the number of cancers among all the positive tests, i.e., true-positives/[true-positives + false positives]) is directly tied to cancer prevalence in the screened population. The lower the prevalence, the lower the PPV. PPV has been very low among traditional tumor markers and has led to their failure as mass cancer screening tests. Among a number of traditional cancer biomarkers (Table 1), we will look at studies of men undergoing asymptomatic prostate cancer screening with prostate-specific antigen (PSA), and women with breast cancer in remission under surveillance with carcinoembryonic antigen (CEA), CA-15.3, and CA-27.9. Although these biomarkers have failed to demonstrate utility, we look ahead to improvements in the PPV with novel imaging techniques in high-risk populations and circulating DNA and tumor cells to identify at-risk patients with greater precision.

### Key Points

- “Screening” is the use of a test to detect cancer sooner (secondary prevention) or prevent its complications (tertiary prevention) among individuals at high risk for cancer. When screening is offered for patients already diagnosed with cancer, the term “surveillance” should be used instead.
- Tumor markers, such as prostate-specific antigen, carcinoembryonic antigen, CA-15.3, and CA-27.29, have not demonstrated accuracy and efficacy in neither diagnosing prostate cancer nor detecting breast cancer relapse sooner than other screening tools.
- Mammography in women age 50 to 70 (even possibly starting at age 40) and colonoscopy in the healthy population over age 50 play an important role in the early detection of cancer and are part of recommended guidelines for cancer screening.
- For patients at high genetic or familial risk for cancer, the addition of breast MRI is recommended for women in their early to mid-20s and colonoscopy is recommended for individuals in adolescence to their mid-20s (based on specific mutation or family history). For patients with a previous or active smoking history, an annual low-dose CT scan can be considered starting in their 50s.
- Circulating tumor cells and circulating tumor DNA offer novel genomic approaches to detect cancer through liquid biopsies and early screening programs, and further studies establishing their clinical utility are now required.

### Prostate-Specific Antigen as an Example for New Diagnosis of Cancer
PSA (kallikrein-3 [KLK3]) is a glycoprotein secreted by epithelial cells of the prostate gland, and is elevated in prostate cancer as well as benign prostatic hypertrophy and prostatitis. Although prostate cancer is an important cause of morbidity and mortality, the PSA test, which is both safe and acceptable, has led to the diagnosis of more cancers at an early age. However, it is not a perfect test, and like other screening methods, it can lead to overdiagnosis, where patients are treated for cancers that would have never required any medical intervention.

### Table 1. Tumor Markers Commonly Used for Screening or Surveillance

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Cancers</th>
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<tbody>
<tr>
<td>Alpha-fetoprotein</td>
<td>Germ cell tumors and hepatocellular carcinoma</td>
</tr>
<tr>
<td>Beta-human chorionic gonadotropin</td>
<td>Choriocarcinoma and testicular cancer</td>
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<tr>
<td>Beta-2 microglobulin</td>
<td>Multiple myeloma, chronic lymphocytic leukemia, and some lymphomas</td>
</tr>
<tr>
<td>CA-125</td>
<td>Ovarian</td>
</tr>
<tr>
<td>CA-15.3, CA2-7.29</td>
<td>Breast</td>
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<tr>
<td>CA-19.9</td>
<td>Pancreas, gall bladder and bile duct, and gastric</td>
</tr>
<tr>
<td>CD20</td>
<td>Non-Hodgkin lymphoma</td>
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<tr>
<td>Calcitonin</td>
<td>Medullary Thyroid</td>
</tr>
<tr>
<td>Carcinoembryonic antigen</td>
<td>Ovarian, cervix, breast, urinary tract, gastrointestinal, and lung</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>Germ cell tumors</td>
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<tr>
<td>Prostate-specific antigen</td>
<td>Prostate</td>
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early stage. In fact, the poorly defined natural history of the disease has simply led screening to detect pseudocancers that would never have been problematic in most men. The positive predictive value (PPV) of the PSA test has been estimated at 30%. The large majority of men who test positive with PSA at over 4.0 ng/mL do not have clinical prostate cancer. Although zero to one man in 1,000 might not die because of PSA screening, 110 would be diagnosed and, if treated, placed at risk of substantial morbidity. Of this group, 29 would develop erectile dysfunction, 18 urinary incontinence, two a serious cardiovascular event, and one deep venous thrombosis or pulmonary embolism. The U.S. Preventive Services Task Force (USPSTF) thus concludes “that there is moderate certainty that the benefits of PSA-based screening for prostate cancer do not outweigh the harms,” and discourages the routine use of this test. The American Society of Clinical Oncology (ASCO) and the American Cancer Society take a more temperate recommendation stating that “men who have at least a 10-year life expectancy should have an opportunity to make an informed decision with their health care provider about whether to be screened.”5,6

Carcinoembryonic Antigen and Other Markers for Detection of Breast Cancer Recurrence

CEA is a set of highly related glycoproteins involved in cell adhesion and is elevated in adenocarcinomas of ovarian, cervical, colorectal, lung, urinary tract, and breast origin. Elevated levels of the cell surface antigens CA-15.3 and CA-27.29 can identify breast cancer recurrence and metastases before symptoms occur. Risk of relapse and mortality from breast cancer is high, these tests are cheap and acceptable, and the natural history of the cancer is well understood, therefore employing these markers seems logical. Moreover, since cancer surveillance involves patients with a high chance of recurrence, that is, high cancer prevalence, these tests have high positive predictive value (PPV) and should have utility. However, use of these markers has not translated to any survival benefit. In serial guidelines, most recently in 2007, ASCO stated that there is insufficient evidence regarding disease-free survival, overall survival, quality of life, toxicity, or cost-effectiveness to support the routine use of CA-15.3 and CA-27.29 in clinical practice. However, a recent study suggested that the use of these markers to predict breast cancer relapse remains a common practice among oncologists. Among oncologists, factors associated with this overuse of surveillance testing include providers who are older age, low provider self-efficacy, international medical graduates, and greater perceptions of ambiguity about survivorship care. Patients who are more likely to be tested serially for these markers are younger at diagnosis, have advanced stage at diagnosis, and reside on the East or West Coast.

**IMAGING AS AN APPROACH TO SCREENING FOR CANCER**

Screening for cancer using radiographic imaging has been available for decades, and multiple clinical studies having demonstrated its efficacy in specific instances. Despite the evidence for the role of imaging as part of an early cancer screening and surveillance program, debate continues about the specific timing and imaging modalities that provide the most benefit with least harm to general and high-risk populations. The three cancers with the best consensus for benefit from early cancer detection using imaging include breast cancer, colorectal cancer (CRC), and most recently, lung cancer.

**Breast Cancer Imaging**

**General population.** In 2014, over 235,000 new cases of breast cancer were diagnosed in the United States and over 40,000 deaths attributed to the disease. Several guidelines exist for screening for early detection of breast cancer in the average-risk, asymptomatic general population. Breast self-examination starting in the third decade of life can be considered part of screening for breast cancer, although clinical breast examination by a health care provider every 3 years is essential. Some organizations discourage breast self-examination because of the risk for increased biopsies and lack of evidence of benefit. Women should be advised to report any breast changes to their health care provider.

Mammography plays a crucial role for early detection of breast cancer. Pace and Keating published an outstanding review that includes a systematic assessment of mammography benefits and risks. Based on over 50 years of published evidence, they concluded that regular mammography screening reduces breast cancer mortality by 19% (nearly 15% for women in their 40s and 32% for women in their 60s). However, the cumulative risk for false-positive results is extremely high at over 60% for a woman who receives 10 years of annual mammograms in her 40s to 50s, and this can lead to increased anxiety, biopsies, and medical expenses. The starting age and frequency of mammography must be balanced with an individual’s risk for breast cancer and an awareness of a high likelihood of false-positive findings.

Mammography guidelines have been proposed by various organizations with clear overlap, but also clear distinctions. Each organization recommends at a minimum that women between the age of 50 and at least 70 should receive mammography at least every 2 years (with consideration of annual screens by some groups starting at age 40). When discussing this topic with patients, Pace and Keating suggest to highlight: (1) mammography is not a perfect screening test, (2) mammography saves lives (five of 10,000 women age 40 to 49, 10 of 10,000 women age 50 to 59, and 42 of 10,000 women age 60 to 69), (3) mammography can overdiagnose and there is potential for false-positives (at least half of women undergoing annual mammography will be incorrectly told they might have cancer over 10 years, and 20% will require biopsy to prove it is not cancer), and (4) informed decision should rely on family history, individual risk, preferences, and expert recommendations.
High-risk populations (hereditary breast and/or ovarian cancer). Lifetime risk for breast cancer for women with hereditary breast and/or ovarian cancer (HBOC) syndrome (BRCA1/BRCA2 germ-line mutations) approaches 40% to 65%, and some women are diagnosed as early as in their 20s. Recommended screening includes mammography plus breast MRI.\textsuperscript{19,20} Compared with mammography, breast MRI offers better visualization of denser breast tissue often found in younger women.\textsuperscript{21–24} In addition, exposure to mammography before the age 30 has been associated with increased risk for breast cancer in women with BRCA1/BRCA2 mutations.\textsuperscript{25} Mammography plus breast MRI in women who are BRCA1/BRCA2 carriers offers comparable survival benefit with prophylactic bilateral mastectomy at age 25 and prophylactic bilateral salpingo-oophorectomy at age 40.\textsuperscript{26} Sensitivity, metastasis-free survival, and overall survival was higher in patients with familial breast cancer treated with MRI compared with mammography-based screening for invasive cancer, but not ductal carcinoma in situ.\textsuperscript{27} Many guidelines now suggest performing an annual MRI at age 25 and then alternating with digital mammography beginning at age 30 so that imaging of the breasts occurs every 6 months.\textsuperscript{16,28}

Colorectal Cancer Imaging

General population. In 2014, nearly 140,000 new CRC cases were estimated to be diagnosed in the United States and over 50,000 deaths were attributed to the disease. Early CRC detection is known to improve clinical outcomes with multiple iterations of surveillance trials throughout the past 4 decades. Adenomatous polyps represent precursors to CRC, and the National Polyp Study in 1978 demonstrated that their removal dramatically reduces CRC risk.\textsuperscript{29–31} Winawer et al later showed in a randomized clinical trial that colonoscopy 3 years after polyp removal was as effective as annual colonoscopy and urged a 3-year interval before colonoscopy with adenomatous polyp removal.\textsuperscript{29} In more recent years, multiple population-based studies using case control, cross-sectional, and cohort designs have demonstrated that CRC risk and mortality are substantially reduced with regular colonoscopy screenings with odds ratios and standardized mortality ratio ranging from 0.23 to 0.71.\textsuperscript{32–38} Additionally, the protective effects of screening using colonoscopy was noted to be more pronounced in the distal versus proximal colon.\textsuperscript{32–34,36–39} U.S. gastroenterologists now offer screening in the general population at 5-year intervals beginning at age 50 and have begun discussing whether 10-year intervals could be effective.\textsuperscript{32,40} The U.S. Multi-Society Task Force on CRC recently updated their guidelines to recommend 10-year intervals for surveillance after negative screening in individuals at average CRC risk.\textsuperscript{41} The National Comprehensive Cancer Network (NCCN) recommends screening for CRC at age 50 if the patient has no family history of CRC and no personal history of adenoma or sessile serrated polyps (SSP), CRC, or inflammatory bowel disease.\textsuperscript{42} NCCN guidelines suggest screening for CRC using colonoscopy, stool-based guaiac/immunochemical testing, or flexible sigmoidoscopy.\textsuperscript{43} If no polyps or hyperplastic/non-SSP smaller than 1 cm are detected at colonoscopy, then rescreening with any modality is recommended in 10 years. If sigmoidoscopy is negative, then rescreening with any modality is recommended in 5 years. If adenoma/SSP is detected, then depending on number, size, and completeness of resection, repeat colonoscopy may be required every 2 to 6 months, 3 years, or 5 years. Results from the first two CRC screening examinations predict future CRC risks.\textsuperscript{43} Despite the widespread adoption of colonoscopy for CRC screening, Samadder et al recently described in a population-based study that 6% of all patients with CRC still developed interval tumors within 6 to 60 months of colonoscopy (associated with higher rate of adenomas and CRC family history).\textsuperscript{44} Three current randomized clinical trials in Europe and the United States are now investigating screening for CRC using colonoscopy and comparing colonoscopy with fecal immunochemical testing (or no screen) with 10–15-year CRC mortality. The reader is referred to the following consensus update by the U.S. Multi-Society Task Force on CRC\textsuperscript{41} and in-depth review article\textsuperscript{42} for excellent summaries of recent trials and discussions of CRC surveillance.

High-risk population (hereditary CRC syndromes). Patients at risk for hereditary CRC benefit the most from a rigorous screening program for early detection and polyp removal. Samadder et al offer an in-depth discussion about the evidence for screening for CRC in patients at high risk.\textsuperscript{45} Specific recommendations are based on the CRC lifetime risk and earliest age of presentation unique to each syndrome. The NCCN offers excellent guidance on this topic organized by specific mutations.\textsuperscript{42,46} For MLH1 or MSH2 mutations (Lynch syndrome/hereditary nonpolyposis colorectal cancer [HNPPC]), colonoscopy is recommended every 1 to 2 years beginning at age 20 to 25, or 2 to 5 years before the earliest colon cancer diagnosis before age 25. For MSH6 or PMS2 mutations (Lynch syndrome/HNPCC), colonoscopy is recommended every 1 to 2 years beginning at age 25 to 30, or 2 to 5 years before the earliest colon cancer diagnosis before age 30. For an APC mutation resulting in familial adenomatous polyposis (FAP), if no symptoms are present, flexible sigmoidoscopy or colonoscopy every 12 months beginning at age 10 to 15 with consideration of prophylactic colectomy once the patient reaches an adult age. For an APC mutation resulting in attenuated FAP, colonoscopy is recommended during the late teen years, then every 2 to 3 years (Samadder et al suggest starting later at age 20 to 25). For MUTYH mutation (MUTYH-associated polyposis [MAP]), colonoscopy and polypectomy is recommended every 1 to 2 years, starting at younger than age 21 if there is a small adenoma burden. For STK11 mutations (Peutz-Jeghers syndrome), colonoscopy and upper endoscopy is recommended every 2 to 3 years starting at the late teens (risk for stomach cancer is nearly 30%). For SMAD4 mutations (juvenile polyposis syndrome), colonoscopy starting at approximately age 15 is recommended and should be continued annually if polyps are detected or every 2 to 3 years if no polyps are identified, with the same strategy for upper endoscopy (risk for stomach cancer is over 20% if multiple polyps are present).
Lung Cancer Imaging
High-risk smoking population. In 2014, more than 224,000 people in the United States were diagnosed with lung cancer and almost 160,000 patients died from lung cancer, making it the deadliest adult cancer. As imaging technology has advanced, so too has lung cancer screening and early detection using annual low-dose CT (LDCT), which has led to both controversy and excitement in the field of early cancer detection. The National Lung Screening Trial (NLST) is the largest randomized clinical trial to be published. It demonstrated a 20% reduction in death in current or former smokers. Six other lung cancer screening trials have been published or are ongoing. Several recent reviews and editorials, as well as other lung cancer screening trials, have been published or are ongoing.60-66 Several recent reviews and editorials, as well as the current screening guidelines, summarize the benefits and harms associated with LDCT. Similar to breast cancer and CRC, several professional societies have offered overlapping, but distinct, recommendations and guidelines on screening for lung cancer (USPSTF, American College of Chest Physicians/American Society of Clinical Oncology, American Association of Thoracic Surgeons, NCCN, American Cancer Society, and American Lung Association).67 The majority of these organizations recommend annual LDCT for high-risk individuals, which includes patients who are age 55 to 79 with a more than 30 pack-year smoking history, former smokers who have quit within the past 15 years, or patients age 50 to 79 with more than a 20 pack-year smoking history who have additional risk factors. Many cost-effectiveness analyses are being modeled for the national adoption of LDCT as the preferred method of screening for lung cancer in current or former smokers. In one study, the heath care expenditures were estimated to reach $1.3 to $2 billion with 50% to 75% screening uptake and $240,000 in additional costs to avoid one cancer death.67 The authors argue that LDCT will prevent over 8,000 annual deaths from lung cancer, but they, and others, recognize that careful cost-effectiveness analyses will be key to understanding the true value of screening for lung cancer.67-69 Nevertheless, the Centers for Medicare & Medicaid Services (CMS) recently announced that Medicare will cover LDCT in current or previous smokers, a move strongly supported by the American Lung Association. Similar to breast cancer, informed decision-making—with understanding of the high likelihood for false-positives (one in five LDCT screening examinations may detect false-positive results, with each LDCT test 20 times more likely to yield a false-positive result than an actual lung cancer) —is key to initiating a lung cancer screening program. Although LDCT now plays a more accepted and arguably standard role in the early detection of lung cancer, the best prevention is still to encourage smoking cessation.

CIRCULATING DNA AND CIRCULATING TUMOR CELLS IN CANCER SCREENING
Recent major advances in our ability to detect and characterize CTCs and cancer-specific (mutated) cell-free, circulating tumor DNA (ctDNA) have introduced the possibility of utilizing either or both as tests to screen for cancer. Particularly attractive is the possibility of simultaneous screening for multiple primary cancers, including tumor types for which no early detection method is currently available. Such a test should also prove complementary to any current standard organ-specific screening tests. Importantly, each of the current tests has multiple limitations, and poor compliance is a consistent challenge as a result of the unpleasant and/or invasive nature of many investigations. However, many obstacles must be overcome before a blood-based screening test, incorporating either CTCs or ctDNA, is proven to make a valuable contribution to early cancer diagnosis.

Liquid Biopsy in the Advanced Disease and Minimal Residual Disease Setting
Both CTCs and ctDNA are yet to be used in routine clinical practice. A major advantage of using CTCs in the management of cancer is that tumor cells can be isolated, which allows for morphologic identification and molecular characterization, whereas ctDNA analysis is currently limited to mutation detection. In advanced disease, both have demonstrated prognostic significance in multiple tumor types, and initial changes in marker levels on therapy also provides an early indicator of treatment response. Serial CTC and ctDNA analysis during therapy can also inform treatment resistance, including ctDNA-based detection of emerging mutations under the selective pressure of targeted therapies. In the context of minimal residual disease, recent larger studies using CellSearch have found no or limited prognostic significance of CTCs across multiple tumor types. In contrast, emerging data from studies of ctDNA for CRC and breast cancer indicate that ctDNA detected after definitive therapy for early-stage disease has powerful prognostic significance.

Potential Role for CTCs and ctDNA in Cancer Screening Specificity. The specificity of any cancer screening test is of critical importance, as false-positive tests create patient anxiety and lead to further investigation with associated financial cost and morbidity. Studies of the U.S. Food and Drug Administration (FDA)–approved CTC testing platform, CellSearch by Veridex, indicate limited specificity with CTCs detectable in 4% to 15% of controls that either have no evidence of disease or benign conditions. Many groups have recently reported improvements in the methods for detection, isolation, and characterization of CTCs that promise greater yield and improved specificity.

The early promise is that ctDNA should be a highly specific test, because mutant DNA appears to be only released into the circulation, via apoptosis or necrosis, once there is an invasive tumor. In addition, and most relevant to the context of screening, more than one mutation should be detected for most tumor types of interest, with two false-positives an exceedingly unlikely finding. For the patient in whom only a single mutation is detected, the ability to readily repeat testing to confirm a positive result is advantageous. A repeat test
could also include an extended panel informed by the initial positive result. For example, where the initial panel reveals a mutated RAS, further testing might reveal an APC mutation in the circulation of the individual being screened. Detecting this APC mutation would provide reassurance that the initial screening result was a true positive, but also indicates the primary source is likely to be a CRC.

Sensitivity. When CTCs are assayed utilizing CellSearch, they can only be detected in about half of advanced cancers, and detection rates in patients with early-stage malignancy are no higher than in people without cancer. ctDNA provides a more sensitive marker since it is present in over 80% of advanced cancers, including in many patients in whom CTCs are not detectable. The approximate 50-fold yield for ctDNA also provides a far greater dynamic range to assess changes over time. Most relevant to screening, ctDNA appears to be detectable in 73%, 57%, 48%, and 50% of early-stage colorectal, gastroesophageal, pancreatic, and breast cancers, respectively, including in 47% of patients with stage I tumors and increasing to 55% and 69% for stage II and III disease. Other groups have also reported high detection rates in small series of patients with early-stage non–small cell lung cancer and breast cancer.

Optimizing and Tailoring the ctDNA Screening Panel
Unlike studies of ctDNA in patients with a known and characterized cancer, screening for an occult cancer will require an extended panel that covers the hotspots of genes frequently mutated in common cancers. The concept and feasibility of such a panel were demonstrated in a recent series in which liquid-based Pap smears were examined for somatic mutations known to accumulate in the cervix after shedding from endometrial or ovarian cancers. A prototype test based on 12 frequently mutated genes in these tumors identified between one and five mutations in all 14 samples from patients with known cancer but unknown molecular profile (12 endometrial cancers, two ovarian cancers). No mutations were identified in samples from 14 women without cancer.

A panel of ctDNA mutations could be defined that would screen for the cancers of most interest in the general community population. Alternatively, a customized panel could be defined for particularly high-risk groups, such as hereditary syndromes. For example, a panel for patients with Lynch syndrome would include screening for gene mutations common in CRC and endometrial cancer. For any screening population, overlapping mutation profiles would mean that patients with any specific mutation would be screened for multiple cancers, including common RAS mutations in the HNPCC panel, which complements screening for CRC using colonoscopy and screening for pancreatic cancer.

Determining Further Investigation
Although ctDNA-based testing is attractive given the broad range of cancers that could be screened for using a single test, this advantage comes with a challenge—determining the source of a positive test. Medical oncologists are familiar with tissue-of-origin molecular profiling, which has an important role in the diagnosis of cancers of unknown primary. For a patient with a positive ctDNA result, a search for a primary tumor is also required, albeit with very different goals.

When ctDNA is detected, further investigation could be guided by the type of mutation(s) found, cancer risk factors, and the relative incidence of each cancer type. Smoking history would indicate an increased yield from lung imaging, and a family history or genetic syndrome would also suggest likely primary sites. Although BRAFV600E mutations are uncommon in CRC (present in approximately 8% of tumors) and ubiquitous in hairy cell leukemia, this mutation is more likely associated with CRC as a result of the relative incidence of the two malignancies.

Alternate search strategies can be envisaged that should prove complementary to an organ-specific approach. CTC analysis should prove increasingly useful with the anticipated improvements in technology, potentially providing further confirmation of a true-positive ctDNA result. CTC detection would also provide opportunity for further characterization of the detected cells, which could guide the hunt for the primary site. Alternatively, whole body imaging, structural and/or functional, may prove to be a viable alternative to an organ-by-organ approach, given the high pretest probability. However, with each new investigation there is discomfort, cost, and the possibility of a false-positive result, therefore the value of each test needs to be carefully defined in prospective studies.

Combining ctDNA with Current Screening Modalities
The most exciting application of ctDNA is creation of a viable screen for many cancers for which no method of early detection is currently available. For tumors such as CRC or breast cancer, ctDNA-based testing should be complementary to the current screening modalities, as each test has a miss rate and is challenged by limited uptake. Importantly, for the 50% or more of patients who fail to comply with colonoscopic screening or the 30% or so who do not undergo regular mammography, it could provide an acceptable initial screening option. ctDNA testing could also prove helpful in characterizing indeterminate findings from routine screening tests, such as small lung lesions found using a screening CT scan in a patient who is a smoker.

OTHER ctDNA CONSIDERATIONS AND CONCLUSIONS
Although stage at diagnosis is the dominant prognostic factor for malignancies, the early diagnosis of a particular cancer type does not necessarily lead to higher rates of cure, and potential risks include overdiagnosis and/or overtreatment of cancers. It is presumed that for each primary cancer there will be a typical window from the point at which ctDNA is initially detectable to when the lesion is incurable, a window that may be only a few months or may be several years, and potentially may vary widely within a particular cancer type. There is much still to be learned.
A multitude of blood-based biomarkers have previously been proposed as cancer screening markers, but none have yet proven to be clinically useful, demonstrating the many challenges of translating initially promising data to a clinical reality. ctDNA-based cancer screening tests would appear feasible, given the available data regarding sensitivity and specificity. From here, carefully conducted clinical studies are required to determine the risks and benefits of early diagnosis across a broad range of tumor types, the optimal frequency of testing, the most desired ctDNA panel, the most efficient algorithms for further investigation of any positive test, and the patient populations that will benefit most from screening.

**ACKNOWLEDGEMENTS**

Joshua D. Schiffman holds the Edward B. Clark MD Endowed Chair in Pediatric Research at the University of Utah and is supported through the Primary Children’s Hospital Pediatric Cancer Program, funded by the Intermountain Healthcare Foundation and the Primary Children’s Hospital Foundation.

**Disclosures of Potential Conflicts of Interest**

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**Employment:** None. **Leadership Position:** None. **Stock or Other Ownership Interests:** None. **Honoraria:** Joshua D. Schiffman, Affymetrix, Inc. **Consulting or Advisory Role:** None. **Speakers’ Bureau:** None. **Research Funding:** None. **Patents, Royalties, or Other Intellectual Property:** None. **Expert Testimony:** None. **Travel, Accommodations, Expenses:** None. **Other Relationships:** None.

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