original report

Multi-Institutional, Prospective Clinical Utility Study Evaluating the Impact of the 92-Gene Assay (CancerTYPE ID) on Final Diagnosis and Treatment Planning in Patients With Metastatic Cancer With an Unknown or Unclear Diagnosis

abstract

Purpose Metastatic cancers of unknown primary or with unclear diagnoses pose diagnostic and management challenges, often leading to poor outcomes. Studies of the 92-gene assay have demonstrated improved diagnostic accuracy compared with standard pathology techniques and improved survival in patients treated on the basis of assay results. The current study assessed the clinical impact of the 92-gene assay on diagnostic and treatment decisions for patients with unknown or uncertain diagnoses.

Methods Patients in this prospective, multi-institutional, decision-impact study included those for whom the 92-gene assay was ordered as part of routine care. Participating physicians completed electronic case report forms that contained standardized, specialty-specific questionnaires. Data collection included patient and tumor characteristics and clinical history. The key study objective of clinical impact was calculated on the basis of changes in final diagnosis and treatment after testing.

Results Data collection included 444 patients, 107 physicians (73 oncologists and 34 pathologists), and 28 sites. Molecular diagnoses from 22 different tumor types and subtypes across all cases were provided in 95.5% of patients with a reportable result (n = 397). Physicians reported that the 92-gene assay was used broadly for diagnostic dilemmas that ranged from single suspected tumor type (29%) to a differential diagnosis of two or more suspected tumor types (30%) or cancers of unknown primary (41%). Integration of 92-gene assay results led to a change in the recommended treatment in 47% of patients.

Conclusion Findings from this clinical utility study demonstrate that the 92-gene assay led to a change in treatment decisions in every other patient case. These data additionally define the role of this assay in clinical practice and strongly support the consideration of molecular tumor typing in the diagnosis and treatment planning of patients with metastatic cancer with unknown or uncertain diagnosis.

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INTRODUCTION

Metastatic cancers that are initially characterized as unknown primary (CUP), or with other features that may lead to diagnostic uncertainty with respect to tumor type diagnosis, can prolong the clinicopathologic workup and result in delays in the initiation of treatment, additional costs, and relatively poor patient outcomes.[1-3]

Novel technologies and therapeutic options to help improve patient outcomes are needed. Numerous diagnostic and treatment strategies, such as enhanced pathologic techniques, molecular classification of tumor type and subtype, comprehensive mutational profiling, and novel combination regimens of cytotoxics plus biologics, have been proposed without universal consensus.[4-10]

Investigational approaches to personalize the systemic treatment of metastatic cancer solely on the basis of the patient's somatic genomic alterations, disregarding histologic context, have had mixed and disappointing results.[11-14] Results from several recent phase II studies that evaluated targeted agents for specific genomic alterations across a variety of tumor types have provided evidence that mutations are not targetable in similar manners across tumor types.[11,12] Thus, the clinical utility of this approach is unclear. Whereas driver mutations matter, the integration of tumor type and subtype remains critical when considering the efficacy of a targeted therapy aimed at a putative driver mutation.[15] Given that empirical chemotherapy approaches are associated with poor prognosis in patients with CUP or uncertain diagnoses, it might be more effective to refocus on methods with which to identify patient tumor type and subtype to guide therapy.[1,16]

Gene expression profiling–based molecular classification of tumors might prove efficacious by helping to identify the primary tumor type and histologic subtype for patients with unknown or uncertain diagnoses. The 92-gene assay (CancerTYPE ID) is a real-time RT-PCR–based assay that utilizes differential gene expression to assign tumors to one of 50 tumor types and subtypes in its spectrum. In clinical studies, molecular tumor classification using the 92-gene assay demonstrated 87% accuracy (95% CI, 84% to 89%), improved diagnostic accuracy compared with standard pathology techniques in poorly and undifferentiated metastatic tumors, and increased overall survival in patients with CUP who were treated on the basis of assay-directed therapy.[16-18] A survey-based retrospective study assessed the clinical utility of the 92-gene assay in decision making in clinical practice, but there have been no large-scale prospective studies to assess its impact in the clinical setting.[19] The current study assessed the clinical utility of the 92-gene assay in patients with unknown or uncertain diagnoses that were submitted as part of routine clinical care in the community setting. The primary objective was to assess clinical impact on the basis of changes in diagnostic and treatment decisions after the incorporation of 92-gene assay results.

METHODS

Study Design

Observational in nature, the current study was prospectively defined to evaluate multidisciplinary clinical utility in patients for whom physicians ordered the 92-gene assay as a clinical workup tool to help identify or narrow the tumor type and subtype diagnosis. Patients were eligible to participate if a biospecimen was available for testing. Study protocol included standardized physician questionnaires and a prespecified analysis plan, and was reviewed and approved by an independent institutional review board. A waiver was granted and informed consent was not required by participating institutions. Prespecified objectives were to examine the diagnostic and clinical utility of the 92-gene assay in oncology and pathology practice to characterize indications of use, and to evaluate its potential integration and impact on patient management by comparing changes in diagnosis and treatment selection after testing.

Data Collection

Physicians were required to complete standardized, discipline-specific questionnaires—pathology and oncology—after receiving a 92-gene assay test report. Physicians entered responses to questionnaires via a secure, Health Insurance Portability and Accountability Act–compliant, Web-based electronic case report form (eCRF). The pathology eCRF consisted of 11 multiple choice questions and four questions that required written responses (biopsy quality, number and types of immunohistochemistry [IHC] performed, and 92-gene assay result). The medical oncologist eCRF consisted of 11 multiple choice questions and five questions that required written responses (time between biopsy and treatment, clinical diagnosis for first-line treatment, name of first-line treatment, other diagnostic or clinical considerations for choosing first-line therapy, and 92-gene assay result). For patient cases with scant tissue or insufficient

RNA quality determined to be quantity not sufficient, in which a molecular prediction could not be determined, physicians completed quantity not sufficient eCRFs that consisted of four multiple choice questions and two questions that required written response (biopsy quality and number of IHC performed). eCRF forms are included in the Data Supplement.

Physicians were instructed on the Web portal and eCRF via a standardized training program. For the pathology questionnaire (Data Supplement: Pathology eCRF Question 7 and Oncology eCRF Question 8, respectively), physicians were instructed to align terminology with the 50 tumor types and subtypes classified by the 92-gene assay. Physicians were required to complete the questionnaires within 2 weeks of the date assay results were made available. No patient-protected health information was collected as part of the eCRF. The testing laboratory was blinded to the working clinical diagnosis from the eCRF.

92-Gene Assay

The 92-gene assay was performed as previously described.[17,20] In brief, the assay—real-time RT-PCR—was performed on isolated total RNA from tumor cells that were enriched by either microdissection or laser microdissection. Assay results were reported if they met the PCR analytical quality control threshold for internal controls—PCR cycling threshold > 30. A prespecified computational algorithm generated probabilities for primary tumor type and subtype on the basis of the degree of similarity of the submitted sample to a reference database of gene expression information from more than 2,000 tumors of known tumor type. The test report provided a prediction of the main tumor type and subtype on the basis of the highest relative probability and any additional tumor types that cannot be ruled out. The report also provided a list of tumor types that could be excluded with 95% confidence.

Statistical Analysis

Statistical considerations of study size were based on an anticipated treatment recommendation change rate of 35%, targeting a sample size of at least 156 patients to ensure a 95% two-sided CI width of 15%. Descriptive statistics were used to summarize the case and patient characteristics. The analytical success rate was calculated on the basis of the proportion of cases with a reportable result. The primary objective of the study was to measure the clinical impact of the 92-gene assay results on the basis of changes in patient treatment, the narrowing of treatment options, or the elimination of a treatment option (“a,” “b”, or “c” on question 15 of the Oncology Questionnaire in the Data Supplement). Patients were considered eligible for a targeted agent if the physician responded “a," "b," or c” in question 15 and there was a US Food and Drug Administration–approved targeted therapy that was recommended by the National Comprehensive Cancer Network for the molecular diagnosis provided by the 92-gene assay.

RESULTS

Patient enrollment began in February 2013 and closed October 2014 after enrolling 444 patients with 107 physicians—73 medical oncologists and 34 pathologists—from 28 sites—21 oncology sites and seven pathology sites—across the United States. Of the 444 patients enrolled, 397 had sufficient RNA for analysis, which corresponded to an overall analytical success rate of 89%, taking into account samples that were determined to have insufficient tissue on pathology review before testing. The primary objective was calculated in patients that received anticancer therapy (n = 203). A patient flow diagram is shown in Figure 1.

Biopsy and Tumor Characteristics

The most common metastatic biopsy sites included the liver (23%), lymph nodes (17%), and lung (14%; Fig 2A). Samples were derived primarily from core needle biopsies (49%) and fine-needle aspirations (11%; Fig 2B). For fine-needle aspiration samples with inherently limited cellularity, the analytical success rate for the assay was 93%. Of the samples for clinical testing that were submitted by pathologists (n = 126), 48% were poorly differentiated or undifferentiated, 29% were moderately differentiated, and 4% were well differentiated (19% were without an assigned grade).

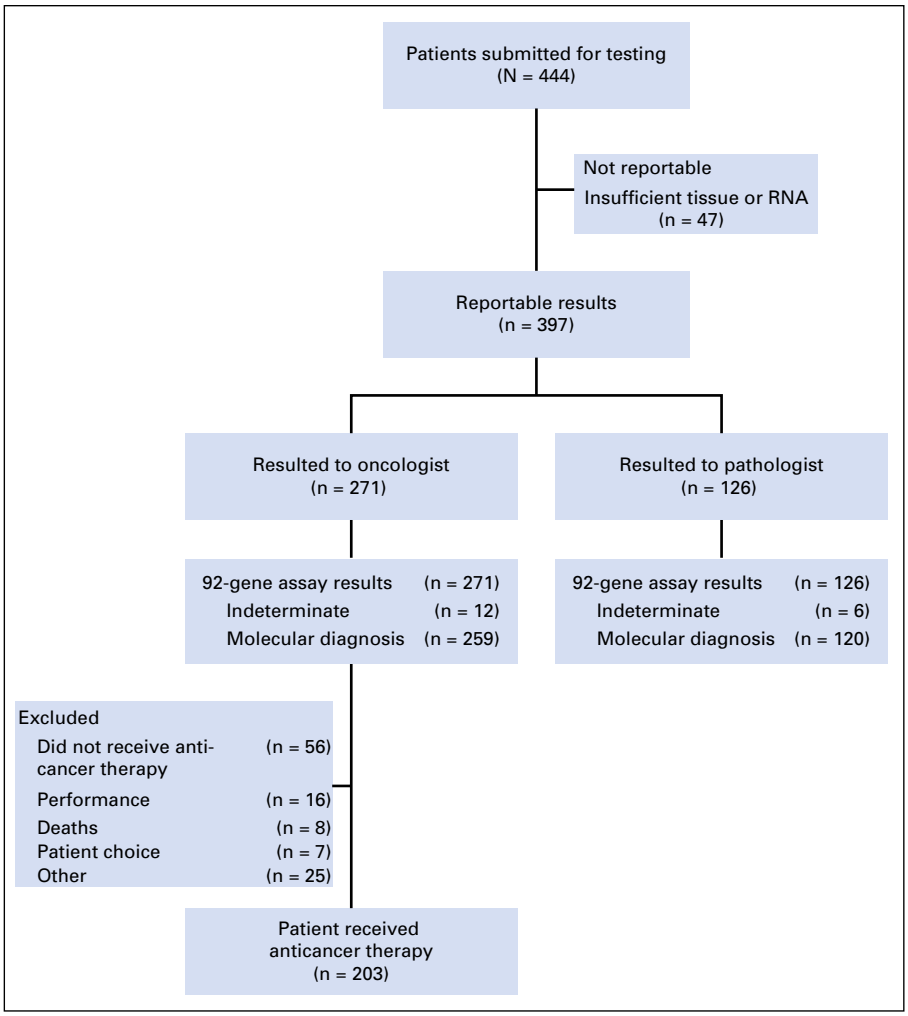

Fig 1. Patient flow diagram showing the disposition of 92-gene assay testing.

Diagnostic Testing and Preassay Diagnosis

Factors that contributed to an oncologist's decision to order the 92-gene assay were multidisciplinary and included the following: no primary site of origin after clinical review and imaging (42%), a pathology report that indicated a differential diagnosis (21%) or that indicated an unknown primary site (20%), and distinguishing between new cancer versus recurrence (16%). Data that were collected to better characterize the sequence of diagnostic testing demonstrated that 72% of physicians responded that patients had pathology and IHC studies performed before the 92-gene assay, 14% of samples were submitted for pathology and IHC evaluation and the 92-gene assay concurrently, and approximately 14% of samples were submitted without an indication of the diagnostic sequence. The most common imaging tests were computed tomography scans (86%), fusion positron emission tomography/computed tomography scans (57%), magnetic resonance imaging (29%), ultrasound (28%), regular film radiographs (11%), or mammogram (11%; data not shown).

For pathologists, inconclusive IHC (50%) was the most common reason for ordering the 92-gene assay. In these cases, 90% were submitted for 92-gene assay testing after the first set of IHC stains were performed. The mean number of IHC stains performed before the molecular assay was ordered was 10 (median, nine; range, zero to 23; data not shown).

92-Gene Assay Results and Impact on Diagnosis

Of the 397 patients with sufficient tissue and RNA for a reportable result, the 92-gene assay provided a molecular-based tumor type and histologic subtype diagnosis in 379 patients (95.5%), whereas 4.5% had an indeterminate molecular diagnosis (Fig 1). Across all submitted cases, the assay predicted 22 different tumor

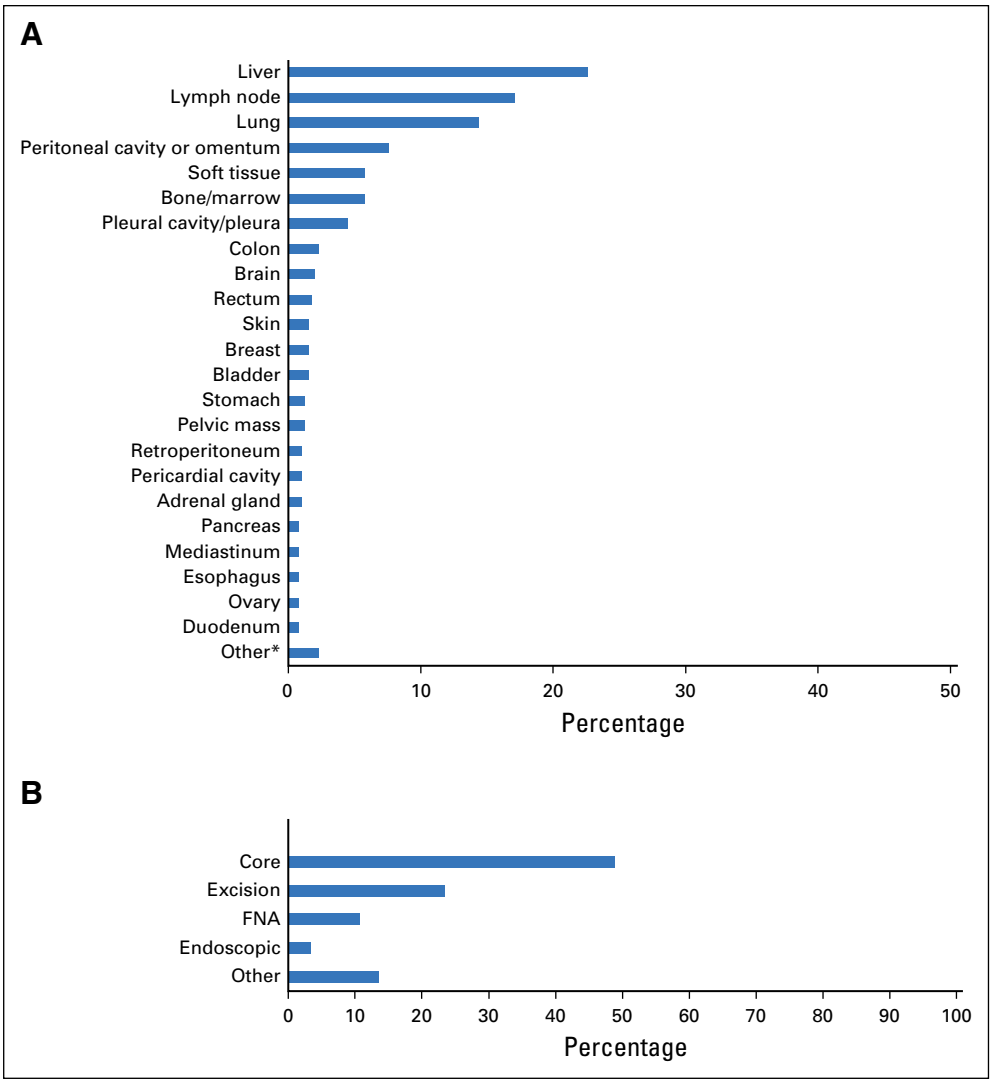

Fig 2. Biopsy sites and biopsy types. (A) Percentage of biopsy sites and (B) biopsy types from 397 total cases. (*) Other indicates biopsy sites with fewer than three cases, encompassing uterus, gallbladder, gastroesophageal junction, spine, spleen, thyroid, and salivary gland. FNA, fine-needle aspiration.

types. The most common diagnoses were pancreaticobiliary (21.9%), squamous cell carcinoma (10.1%), lung adenocarcinoma (9.3%), and intestinal (8.6%) type tumors (Fig 3).

Working diagnoses before the 92-gene assay were assessed in the medical oncology subset (n = 271). Preassay working diagnoses were reported as a single suspected site in 79 patients (29%), a differential diagnosis of two or more suspected sites in 80 patients (30%), and CUP in 112 patients (41%; Fig 4A). Comparison of the 92-gene assay molecular diagnoses with preassay working diagnoses demonstrated that the assay provided a tumor type diagnosis that was not initially suspected in a large proportion of patients (Fig 4B). In patients with a single suspected primary site (n = 79), the 92-gene assay confirmed the suspected diagnosis in 60% of patients but provided a tumor type result that was not initially suspected in 39% of patients. In patients with a differential diagnosis (n = 80), the 92-gene assay narrowed the diagnosis in 66% of patients and provided a tumor type result that was not initially suspected in 27% of patients. In patients for whom the pathology report indicated CUP site (n = 112), the assay provided a molecular tumor type prediction in 97% of patients.

Impact on Treatment Planning

Of the 271 oncologist-submitted cases, 203 patients (75%) received anticancer treatment after the 92-gene assay results were made available. The most common reasons for patients not receiving treatment were a rapid deterioration in the patient's performance status (29%), patient death (14%), and a patient declination of treatment (13%; Fig 1). After receiving the results of the 92-gene assay, medical oncologists modified their treatment recommendation in 47% of cases

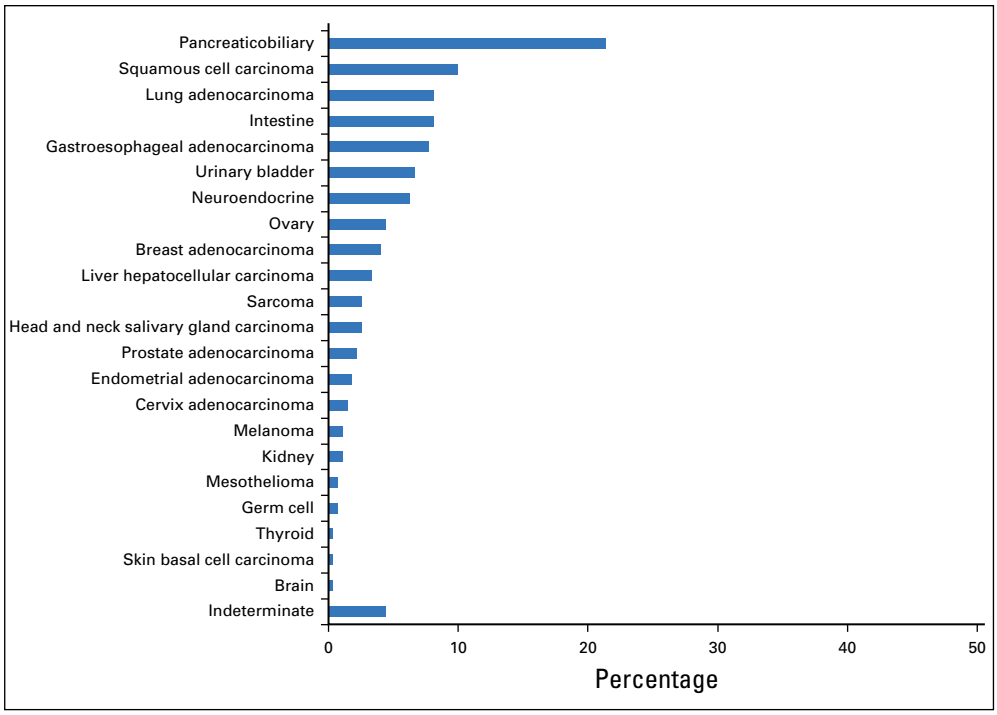

Fig 3. Molecular diagnosis provided by the 92-gene assay. Molecular cancer classification predictions from submitted cases using the 92-gene assay (n = 397).

(Fig 5A). The assay resulted in similar changes in treatment recommendations in all three scenarios of the original working diagnosis—48% of cases with a single suspected primary (n = 79), 49% of differential diagnoses with two or more primaries (n = 80), and 42% of cases with an unknown primary site (n = 112).

Subset analysis within the most commonly predicted tumor type classes—those with ≥ 20 cases—demonstrated that physicians changed their recommended treatment in 58% of GI cancers (n = 81), 54% of gynecologic and breast cancers (n = 28), and 44% of lung cancers (n = 27; Fig 5B). Molecular biomarker testing for somatic mutations—for example, *EGFR*, *ALK*, and *KRAS*—was ordered in 38% of cases and was primarily dependent on tumor type. Mutational biomarker testing was most commonly ordered after the 92-gene assay rendered a diagnosis of lung (81%) or colorectal cancer (67%).

DISCUSSION

Results from this large, multisite study have demonstrated that the 92-gene assay was used across a spectrum of diagnostic uncertainty, beyond CUPs, and included cases with differential diagnoses and those with a suspected diagnosis for confirmatory testing. The assay provided a molecular tumor type prediction in 97% of unknown primary cases with sufficient tissue for testing. In addition, the assay provided a diagnosis that was not initially suspected in a substantial proportion of patients who had either a single suspected diagnosis or differential diagnosis before testing with the 92-gene assay. The key finding of the study was that the incorporation of the 92-gene assay results led to changes in treatment recommendations in 47% of patients. This clinical impact was more pronounced in GI and gynecologic and breast tumor types, which suggests that the 92-gene assay may have additional utility in particular metastatic presentations in which standard approaches may be limited. Moreover, the impact on treatment recommendations was similar regardless of whether the preassay working diagnosis was CUP, a differential diagnosis, or a single suspected primary site. Twenty-four percent of patients whose treatment decisions were changed after the integration of the 92-gene assay results received targeted therapy. Given the 30,000 to 50,000 new cases of CUP in the United States every year,[1,21,22] as well as a significant number of additional patients with some level of diagnostic ambiguity regarding tumor type or subtype, the implementation of molecular-based diagnostic assays may provide significant clinical impact.

A critical question that has been posed in clinical oncology practice in recent years has been, in the age of precision medicine, comprehensive mutational profiling, and targeted therapy

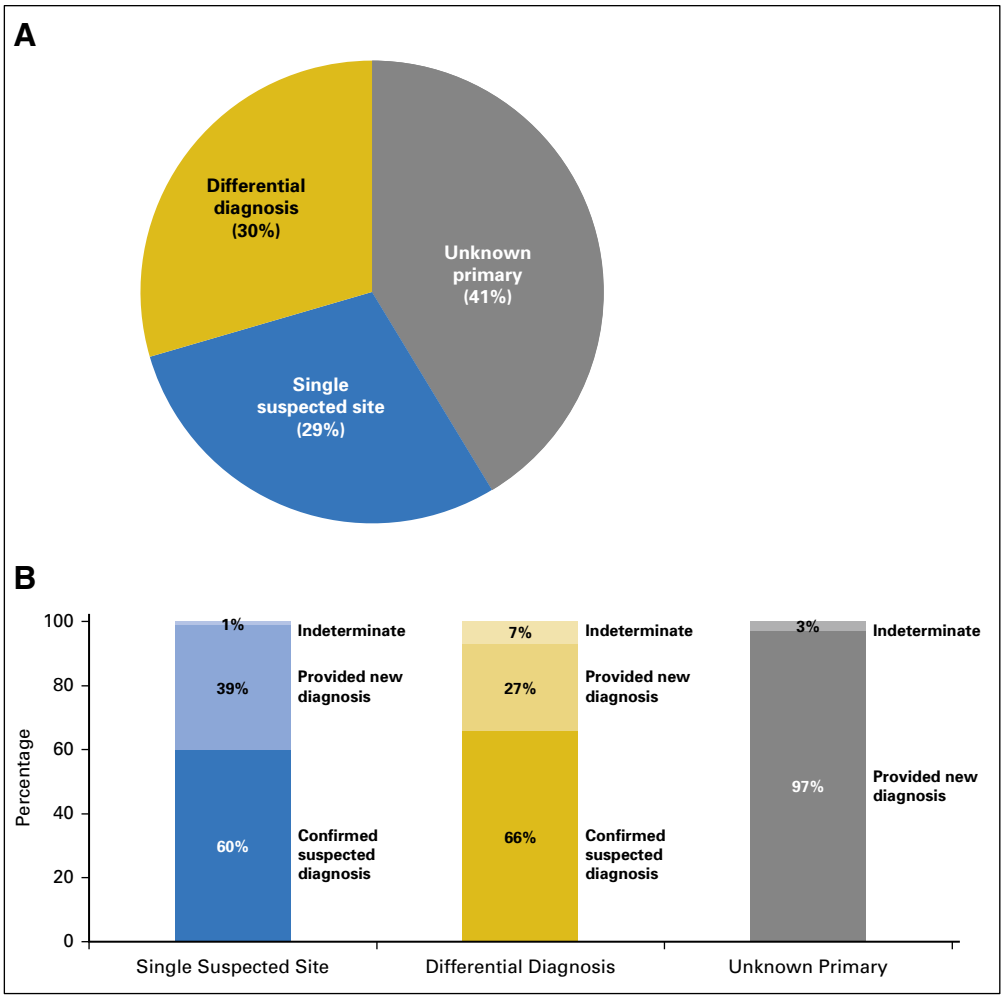

Fig 4. Impact of 92-gene assay on clinical diagnosis and treatment decisions. (A) Oncologist pre–92-gene assay working diagnosis showing clinical use across a spectrum of diagnostic uncertainty (n = 271). (B) Characterization of 92-gene assay diagnoses within preassay working diagnosis. Distribution of 92-gene assay diagnostic results within the preassay working diagnosis classifications.

approaches, what is both the current and future relevance of establishing the tumor type or cellular context of a metastatic cancer? Recent results from a number of basket trials have demonstrated that knowledge of tumor type and cellular context remains fundamental for the interpretation of potentially targetable DNA mutations and the recommendation of treatment approaches in metastatic cancer. Whereas molecularly targeted agents have been demonstrated to be effective in tumors that harbor a matching molecular alteration, a growing understanding of the importance of molecular heterogeneity and cellular context is emerging. For example, the efficacy of the targeted BRAF inhibitor, vemurafenib, has been shown to be mixed across a diverse set of nonmelanoma cancers with a *BRAF* V600 mutation[11] and is well known to have poor efficacy in *BRAF*-mutated colorectal cancers.[11,13] Similarly, early results from the MyPathway basket trial have demonstrated variable response rates in patients with identical mutations across different tumor types.[14] Finally, in a phase II study in which patients with a specific molecular alteration were randomly assigned to receive treatment with a molecularly targeted agent or physician's choice of treatment, there was no difference in median progression-free survival between the two treatment groups.[12] These data suggest that the effectiveness of targeting putative driver mutations with molecularly targeted agents may be dependent on the specific cellular context or tumor type. Results of larger basket trials, including the ASCO TAPUR and NCI-MATCH trials, will help to further clarify the effectiveness of this approach and define the interplay of genomic alterations and cellular context. The importance of cellular context and tumor type has also been reported for the management of liver metastases by using stereotactic body radiotherapy. Colorectal adenocarcinoma histology has been found to be more radioresistant compared with other tumor types and histologies, including anal squamous cell cancer,

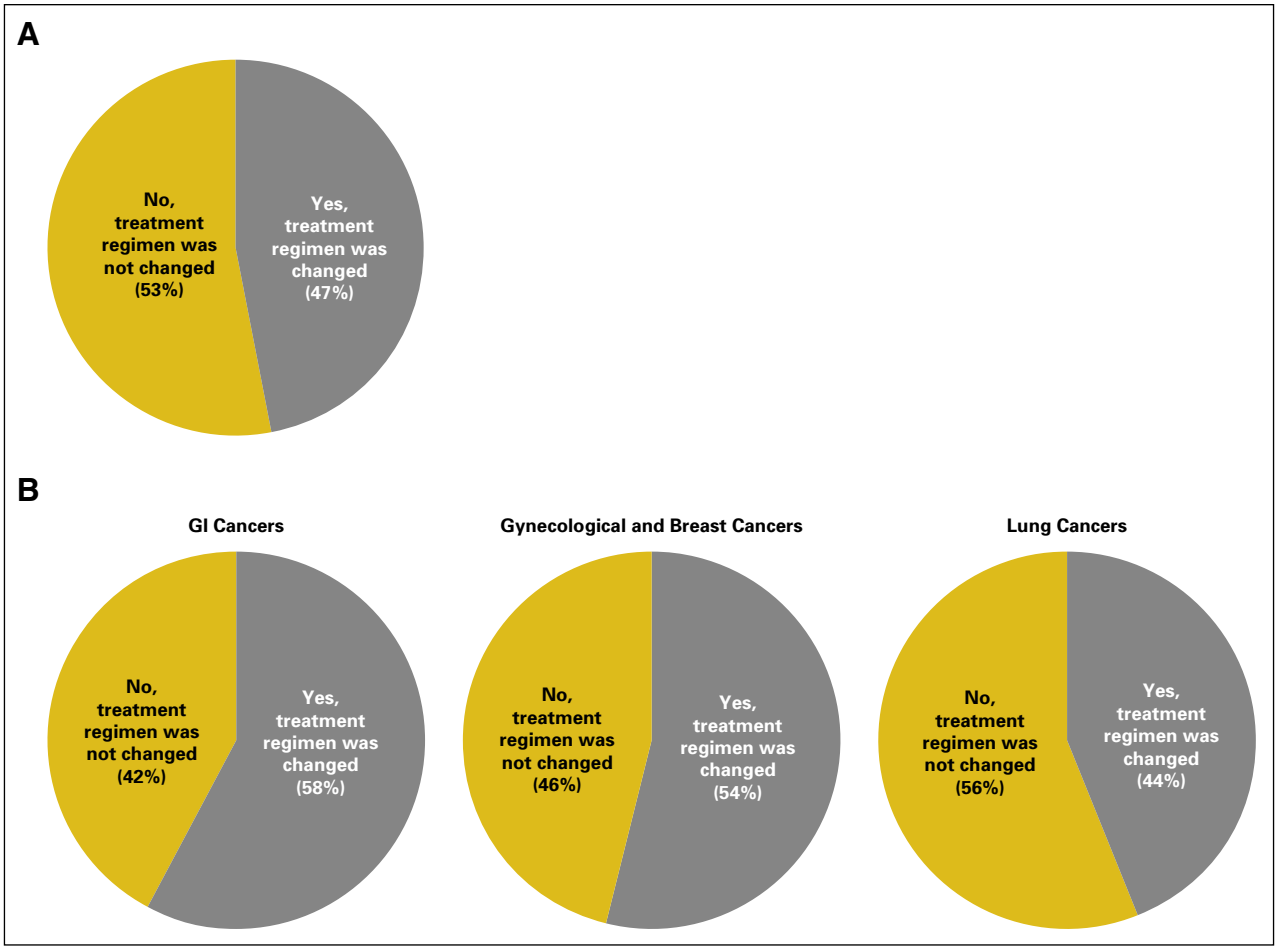

Fig 5. (A) Percentage of patients in which the 92-gene assay affected the recommended treatment decision (n = 203). Patients who died and/or did not receive therapy were excluded. (B) Impact of the 92-gene assay on treatment decisions within tumor type subgroups that received therapy: GI cancers (n = 81), gynecologic and breast cancers (n = 28), and lung cancers (n = 27).

breast adenocarcinoma, and lung adenocarcinoma.[23-25]

Results presented here reinforce the continued relevance of tumor type diagnosis in optimizing treatment strategies that can potentially affect patient outcomes. Findings from the current study compare favorably with other studies that have used either multiplatform—IHC, next-generation sequencing, fluorescence in situ hybridization—tumor profiling[26] or microarray-based tumor classification.[27] The most common main tumor types predicted by the 92-gene assay were pancreaticobiliary (21%), squamous cell carcinoma (10%), lung adenocarcinoma (8.1%), and intestinal (8.1%), gastric (7.7%), and bladder (6.6%) tumors. These tumor types are common putative primary sites for CUP,[16,28] but have distinct recommended first-line, site-specific systemic therapy approaches. With regard to pancreaticobiliary tumors, the 92-gene assay also reports additional subtyping into gallbladder adenocarcinoma, pancreatic adenocarcinoma, or cholangiocarcinoma, which may inform treatment decisions for surgery type, neoadjuvant chemotherapy, or site-directed and targetable agents. In addition, a significant proportion of tumor types predicted by the 92-gene assay, including lung adenocarcinoma, lung squamous, colorectal, gastroesophageal, urinary bladder, and neuroendocrine tumors, have not only specific chemotherapy approaches, but also US Food and Drug Administration–approved molecularly targeted therapies or immunotherapies. Immunotherapy approaches with checkpoint inhibitors have variable response rates depending on tumor type.[29-31] Moreover, programmed death ligand-1 biomarker testing can have varying cutoffs on the basis of tumor type.[32,33] These data underscore the continued importance of identifying tumor type and subtype diagnosis when implementing a precision medicine approach to optimize therapeutic strategies and patient outcomes.

Results from this prospective study are consistent with previous retrospective analyses of the 92-gene assay on diagnosis and treatment

decision making, with similar overall results, which supports the clinical utility of the assay.[34,35] Strengths of this study include the large number of patients and contributing physicians, which contributed to the generalizability of the study results. Several limitations should be considered when evaluating the results from this study. First, this was an observational study and no data on outcomes were collected; however, previous studies have demonstrated that the use of the 92-gene assay improved survival in patients with CUP who were treated on the basis of assay results.[16,34] Although multiple aspects of the study design were prespecified and carefully planned, such as study physicians independently completing questionnaires, a blinded design such that the testing laboratory did not have knowledge of working diagnoses, and a preplanned statistical analysis, bias is an inherent feature of observational studies. Finally, participating oncologists and pathologists largely were from different institutions; thus, interdisciplinary interactions were not evaluated in this study. This aspect of the cross-functional integration of molecular diagnostic results warrants investigation in future studies.

This study demonstrated that the 92-gene assay affected diagnosis and treatment selection in a significant proportion of patients, which supports the clinical utility of the assay as a standardized molecular approach to help streamline additional diagnostic testing in patients with metastatic cancer with unknown or uncertain diagnoses. Use of a tissue-sparing molecular assay also may allow for complementary mutational profiling. This approach has been proposed in a diagnostic algorithm that integrates standard-of-care IHC, molecular tumor classification, and comprehensive genomic profiling to maximize the clinically meaningful benefit in patients with CUP.[10] Gene expression–based molecular diagnostic assays have also been incorporated in clinical practice algorithms for patients with CUP.[5] Successful implementation of an integrated and standardized methodology for patients with metastatic cancer with unknown or uncertain primary site may result in a more comprehensive and effective diagnostic approach for optimized treatment planning, including site-specific chemotherapy, molecularly targeted therapy, radiotherapy, eligibility for tumor-specific clinical trials, and immunotherapies with curative potential for a subset of patients with specific tumor types.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Appendix

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