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NCCN Task Force Report: Evaluating the Clinical Utility of Tumor Markers in Oncology

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Kristina M. Gregory, RN, MSN, OCN, is our nurse planner for this educational activity.

Target Audience

This educational program is designed to meet the needs of oncologists, advanced practice nurses, and other clinical professionals who treat and manage patients with cancer.

Educational Objectives

After completion of this CME/CE activity, participants should be able to:

- Distinguish between a prognostic factor and a predictive factor as they apply to oncology
- Define the term "clinical utility" as it applies to the incorporation of tumor markers into clinical practice guidelines
- Describe the factors considered in the evaluation of the clinical utility of a tumor marker in oncology
- Describe the complementary roles played by pathologists and clinicians in ensuring accurate testing of tumor markers
- Explain the difference between the terms "laboratory certification" and "laboratory accreditation" as they apply to pathology laboratories performing tumor marker testing
- Describe the importance of pathologic expertise, internal quality control standards, and external quality assurance monitoring in determining the reliability of tumor marker testing
- Explain the significance of FDA approval as it applies to a diagnostic test
- Summarize initiatives for transitioning basic and translational research findings on tumor markers into clinical practice

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Key Words

NCCN, tumor marker, biomarker, analytic validity, clinical validity, clinical utility, predictive marker, prognostic marker, companion diagnostic marker, molecular marker

Abstract

The molecular analysis of biomarkers in oncology is rapidly advancing, but the incorporation of new molecular tests into clinical practice will require a greater understanding of the genetic changes that drive malignancy, the assays used to measure the resulting phenotypes and genotypes, and the regulatory processes that new molecular biomarkers must face to be accepted for clinical use. To address these issues and provide an overview of current molecular testing in 6 major malignancies, including glioma, breast cancer, colon cancer, lung cancer, prostate cancer, and acute myelogenous leukemia, an NCCN Task Force was convened on the topic of evaluating the clinical utility of tumor markers in oncology. The output of this meeting, contained within this report, describes the ways biomarkers have been developed and used; defines common terminology, including prognostic, predictive, and companion diagnostic markers, and analytic validity, clinical validity, and clinical utility; and proposes the use of a combination level of evidence score to aid in the evaluation of novel biomarker tests as they arise. The current state of regulatory oversight and anticipated changes in the regulation of molecular testing are also addressed. (*JNCCN* 2011;9[Suppl 5]:S1–S32)

The science and practice of oncology are rapidly evolving based on more complete knowledge regarding cancer genomes and specific genetic events driving malignant disease. This knowledge, coupled with advances in medicinal chemistry, has ushered in an era of molecular oncology care in which, increasingly, best care practices require molecular insight. Although tumor markers previously played a role in a relatively small fraction of patients diagnosed with cancer, actionable molecular assays are now available that may guide treatment decisions for the most common, deadly malignancies. Given the rapidity with which the field is changing, NCCN assembled a task force to help educate the community with respect to the terminology of contemporary tumor markers and provide the current state of biomarker validation for the most common forms of cancer.

Although biomarker discovery is thriving, incorporation of biomarkers into clinical practice lags behind. Some of the challenges to clinical adoption include assay variability and inadequate analytic validation; poor study design and analysis; and inadequate reporting,[1–7] and practical obstacles such as lack of resources, personnel, or expertise in smaller clinical laboratories. In addition, a growing number of multi-analyte tumor markers require comprehensive technologies and computational algorithms. These platforms and analytic approaches may be understandable to few practitioners and represent challenges to independent validation and verification. It is imperative that the field of oncology works with a common language and clear standards of evidence so that the merits of established and emerging tumor markers can be communicated in a clear and unambiguous manner.

This report provides the basic definitions for biomarkers in oncology, briefly reviews the steps necessary for the clinical development of a biomarker, and sum-

marizes a previously published approach to categorizing the strength of evidence supporting the use of a biomarker in a specific clinical situation. The task force hopes that this information will be useful to investigators who are discovering and/or validating new biomarkers and molecular tests, and to clinicians, guideline developers, and other stakeholders, such as health policymakers and payors, who participate in decision-making regarding the incorporation of novel biomarkers into clinical oncology. The final section of this report uses examples from 6 important disease areas to illustrate both the merits of tests that are currently in clinical use and the evidence that must be assembled for other tests to be accepted for general use. Through analyzing these examples of established and emerging biomarkers, the task force hopes to codify the appropriate evaluation of the required level of evidence necessary for making recommendations for incorporating new tests into clinical practice guidelines and general clinical use.

Types of Molecular Markers

Molecular markers are developed to address a variety of indications. An individual marker may serve more than one purpose and thus can fall into more than one category of biomarker. In addition, a single biomarker may have different categorization across tumor types and/or stages of disease. This section defines common terms used to describe molecular tests.

Diagnostic Markers

A large category of molecular tests aid in the diagnosis or subclassification of a particular disease state. Diagnostic subclassification may result in different management of the disease, but the marker is used primarily to establish the particular disease that is present in the patient sample. Examples of diagnostic molecular tests include immunophenotyping in non–Hodgkin's lymphoma, and fluorescence in situ hybridization (FISH) to show the presence of the Philadelphia chromosome in chronic myelogenous leukemia.[8]

Prognostic Markers

Prognostic markers have an association with some clinical outcomes, such as overall survival or recurrence-free survival, independent of the treatment rendered.[5] An example of a prognostic marker is the presence of *p53* mutations, which identify subsets of patients who will have a more aggressive disease course for certain cancers, regardless of current treatment options.[9]

Predictive Markers

Predictive markers predict the activity of a specific class or type of therapy, and are used to help make more specific treatment decisions. They are used as indicators of the likely benefit of a specific treatment to a specific patient.[5] Human epidermal growth factor receptor 2 (*HER2*[*ERBB2*]) is one example of a predictive marker. *HER2*(*ERBB2*)-negative tumors do not respond to trastuzumab, and therefore *HER2*(*ERBB2*)-positivity is predictive of potential trastuzumab response in a patient with newly diagnosed breast cancer.[10–12]

Companion Diagnostic Markers

Companion diagnostic markers may be diagnostic, prognostic, or predictive, but are used to identify a subgroup of patients for whom a therapy has shown benefit. Specifically, prospective data show that patients with positive markers benefit from the therapy. Thus, although companion diagnostic markers are primarily a subgroup of predictive markers, evidence may not be sufficient to determine whether they have independent prognostic or predictive strength for the disease or class of therapy. One recent example of co-approval of a drug and companion diagnostic is the *BRAF* V600E mutation test coapproved with the kinase inhibitor vemurafenib. *BRAF* mutations are found in 30% to 60% of melanomas, and the kinase activating *BRAF* V600E mutation confers sensitivity to vemurafenib, a small molecule BRAF inhibitor.[13,14] Package insert material for the drug specifies that a test must be performed to establish whether the tumor carries a *BRAF* V600E mutation.[15]

Types of Validation

For a test to become generally useful, it must have demonstrated analytic validity and clinical utility. The former includes its reproducibility and quality as a test. Clinical validity implies that the marker identifies 2 groups that can be distinguished biologically and have different outcomes, but this observation may not indicate that it should be used for routine clinical care. Clinical utility implies that high-level evidence shows that use of the marker improves patient outcome sufficiently to justify its

incorporation into routine clinical care. This section reviews the terms *analytic validation*, *clinical validation*, and *clinical utility*.

Analytic Validation

Analytic validation focuses on determining how accurately and reliably the assay measures the molecular event of interest.[5,7,16] Assays used in a research setting may be extremely reliable in the hands of skilled and meticulous laboratory investigators, but not scalable or feasible in a general clinical setting with routinely available tissue samples. Even assays that are routinely performed in the laboratory require analytic validation within a clinical laboratory setting to be used in making clinical decisions. Significant differences exist between research laboratories and clinical laboratories, and any assay being developed for the management of patients must be established within a clinical laboratory. For example, immunohistochemistry on formalin-fixed paraffin-embedded (FFPE) tumor samples is a well-established technique widely used in research and essential clinical cancer testing, such as in estrogen receptor 1 (ER-α[ESR1]) and progesterone receptor(PgR[PR]) or HER2(ERBB2) staining in breast cancer. However, results can be influenced by a variety of factors, both within and outside the control of the pathology laboratory. One step that is often neglected is adequate validation of commercial or other antibodies.[17] In one case, a commercial antibody presumably directed against telomerase was subsequently shown to recognize nucleolin.[18]

To ensure reproducible findings, clinical laboratories should understand the impact that preanalytic variables and specimen processing have on assay performance. Simply stated, analytic validation ensures that the same answer will be produced for the same sample within predefined technical variation. Analytic validation does not signify that the result itself has any clinical relevance.

In recognition of the critical importance of analytic validation for biomarkers, multiple groups have developed recommendations and frameworks with which to standardize the assessment. One effort designed to provide a framework to record and potentially control some of the preanalytic specimen–handling variables is the Biospecimen Reporting for Improved Study Quality (BRISQ) recommendations, which resulted from a committee formed at the Biospecimen Research Network Symposium in 2009.[19] Input was received from a wide range of experienced scientists, clinicians, pathologists, statisticians, and professionals from other disciplines, and the resulting publication can be used to assemble a checklist of elements that should be reported for clinical biospecimens, including information about the patient, diagnosis, tissue handling, fixation times, and storage and transport of tissue samples. Although not all information may be available or appropriate for each specimen type, use of a checklist and recommendations such as these can help to ensure that test results can be properly interpreted,[19] whether the samples are used for immunohistochemistry, FISH, mutation testing, or multigene expression assays.

Clinical Validation

Clinical validation assesses the strength of association between the assay results and the clinical outcome of interest, whether it is diagnostic, prognostic, or predictive. A large number of measures are used to assess these associations. Although measures of statistical significance are important, robust measures of the strength of association, such as receiver operator characteristic (ROC) curves, the area under the curve of ROC analysis, and the sensitivity, specificity, and positive predictive value of assays, are critical to understanding the strength of their clinical association and help bridge analysis to clinical utility (discussed later). These analyses address whether one can be sure the clinical state is positive if the test is positive (positive predictive power), and vice versa (negative predictive power).

No single best design exists for validation studies, but some general observations can be made. First, biomarkers are often developed within a single dataset and resampling methods, such as leave-one-out cross-validation, fold-validation, or bootstrapping, are used to set assay parameters and estimate performance. Although these approaches help determine if a stable assay can be developed, they often underestimate the true error rate. For larger datasets, identifying a priori a "discovery" or "training" subset of samples and a "validation" or "test" subset of samples is common practice. The assay's characteristics are tested and refined in the discovery subset and, when the assay parameters are fixed, applied to the validation subset. Although this minimizes bias and overfitting within the dataset, a concern remains that characteristics specific to the dataset being used may result in validation that does not generalize to

other sample or patient populations. Thus, independent validation sets are required for the most rigorous clinical validation of an assay.

Evaluation of new biomarkers can be aided if tumor biomarker studies and the journals reporting them adhere to the Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK). These guidelines were produced by a workgroup convened as part of the NCI-EORTC joint meeting on Cancer Diagnostics in Nyborg, Denmark, in 2000.[20] REMARK criteria include specification of patient populations, biological specimen under study, assay methods, study design, and statistical methods, and detailed guidelines for analysis and presentation of data. This reporting standard is now requested for manuscripts being submitted to journals such as the *Journal of Clinical Oncology*. Although the tissue resources are not always available to allow the most rigorous validation of assays, providing a standardized means to communicate the level of clinical validation is critical to biomarker development and underpins the motivations behind the levels of evidence discussed below. Using reporting standards such as these should help establish the clinical validity of new biomarkers being used in cancer research and treatment.

A good example of rigorous clinical validation is seen in a publication focused on validating several independently derived gene expression–based prognostic markers for early-stage lung cancer.[21,22] In this study, 442 samples were collected from 6 institutions and processed at 1 of 4 laboratories using a single, reproducible protocol, and data were analyzed using a blinded validation step.[21] Previous gene expression studies have been hampered by small sample size, samples having been collected from only a single institution, and lack of uniformity in sample processing and data analysis.

Clinical Utility

Clinical utility refers to the ability of the assay to improve clinical decision-making and patient outcomes. Clinical utility depends on the clinical situation, availability of effective therapies, magnitude of clinical benefit (or lack thereof) in one marker-designated group versus another, and relative value the patient, caregiver, and society place on the differences in benefits and risks in these separate groups, and their perceptions of these differences. For example, even if a marker clearly distinguishes outcomes between "positive" and "negative" groups, if evidence for differential treatment is not available, then no reason exists to test for the marker. Likewise, the marker may separate outcomes of 2 groups, but if the therapy is very effective in both, just slightly less effective in one, the marker does not have clinical utility.

An emerging standard for the adoption of new molecular tests is the demonstration of clinical utility. A novel assay may have outstanding analytic validity and proven clinical validity but may strongly correlate with an established clinical or histopathologic predictor, and thereby provide no additional clinical utility. In prostate cancer, Gleason sum remains the single most prognostic feature of localized cancer. Although many molecular assays have also been found to be associated with prognosis in prostate cancer, none are in broad use because most offer little to no independent prognostic information over Gleason sum and thus lack clinical utility.[23]

Regulation of Molecular Diagnostic Tests

The regulation of molecular testing falls under the jurisdiction of 2 federal agencies—the FDA and the Centers for Medicare and Medicaid Services (CMS). The laws and policies concerning the development and implementation of molecular tests continue to evolve. This section briefly reviews the key elements of the regulatory environment. NCCN also assembled a Molecular Testing Working Group in 2011, which generated a White Paper detailing the regulatory environment and highlighting additional challenges to the development and implementation of biomarkers.[24]

FDA

Medical tests are regulated by the Center for Devices and Radiological Health, a different part of the FDA from that which regulates drugs (Center for Drug Evaluation and Research) or biologics (Center for Biologics Evaluation and Research), leading to regulatory differences that impact FDA evaluation and approval. Regulatory evaluation of medical devices began with the 1976 Medical Device Amendment to the 1938 Federal Food, Drug, and Cosmetic Act, enacted in response to deaths and injuries attributable to some medical device products.[25] Devices are divided into 3 classes based primarily on the risks associated with their intended use. For in vitro di-

agnostic tests, the risks are framed in terms of the consequences for patients when a test renders a wrong result. Class I tests are low risk, usually of a simple design, and typically are exempted from review by the FDA before proceeding to market. Class II tests pose moderate risk, are evaluated by the FDA through review of a 510(k) premarket notification, and are cleared for marketing once they are found to be substantially equivalent to a legally marketed device that was previously cleared by the FDA. Class III tests are those associated with the highest clinical risk. Each Class III test is reviewed through application for premarket approval (PMA). A demonstration of safety and effectiveness, which (for in vitro diagnostic devices) rests largely on a demonstration of analytic and clinical validity, is needed to gain FDA approval. The FDA approval/clearance process provides reasonable assurance of safety and effectiveness (i.e., that the benefit from using the test will outweigh the risk), and that the test will provide clinically significant results. The device regulations do not require a complete evaluation of clinical utility; hence, aspects such as cost-effectiveness or comparative effectiveness are not part of premarket review. A Class III device or test that has been approved for marketing through the PMA process is called *FDA-approved*, whereas a Class II device that has undergone premarket notification review through 510(k) is referred to as *FDA-cleared*.

The rapid translation of new scientific knowledge to medical practice has led to development and use of diagnostic tests that are often called *home brew tests* or *laboratory developed tests* (LDTs). Although the FDA retains jurisdiction over this category of test, until recently it practiced a policy of enforcement discretion for LDTs, choosing instead to regulate the critical reagents that are used to build these tests through the Analyte Specific Reagent Rule[26] (21 CFR 864.4020, 809.10, and 809.30). Enforcement discretion has allowed more tests to be developed and performed, but has also come under recent scrutiny because of the lesser requirements for validation and reporting. The FDA recently released a preliminary guidance on Companion Diagnostics,[27] or molecular tests which directly impact the use of a pharmaceutical or biologic drug, and the agency is expected to take a more active role in molecular test governance as more complex tests begin to enter clinical practice and are used to direct patient care. Importantly, for trials using Class III molecular assays to guide therapy, investigators are now often required to file an investigational device exemption with the FDA before initiating the trial. This relatively recent additional regulatory requirement ensures that acceptable standards of analytic validity are in practice but also places increased regulatory burden on investigators.

CMS Clinical Laboratory Improvement Amendment

Clinical laboratories are governed through the Clinical Laboratory Improvement Amendment (CLIA) of 1988 administered by CMS. *CLIA certification* is a descriptor that is frequently associated with analytically valid assays performed in a clinical laboratory and/or a laboratory that has received CLIA certification. Importantly, individual clinical laboratory assays are not CLIA-certified. Rather, CLIA certification requires that a laboratory adopt specific practices and perform prescribed measures of analytic validation while performing specific assays. CLIA certification is required for laboratories performing low-complexity waived tests and moderate- or high-complexity tests on clinical samples, whether they are FDA-approved, FDA-cleared, or laboratory developed. It has become clear that assays must be performed in CLIA-certified laboratories if the results of the assays are going to be used to guide patient management, regardless of FDA status. CLIA certification prescribes a certain level of analytic validation, but performance in a CLIA-certified laboratory does not address the clinical validity or clinical utility of an assay. However, FDA clearance or approval of a marker has been mostly based on analytic and clinical validity, but not necessarily clinical utility. Therefore, FDA approval does not necessarily mean that a marker should be used, and lack of FDA approval does not mean it should not be used, because an assay with clinical utility can be performed in a CLIA-certified laboratory without FDA approval. Key components of the regulation and oversight of CLIA-accredited laboratories are inspections and proficiency tests. In most jurisdictions and hospitals, these are provided by the College of American Pathologists (CAP). CAP inspection guidelines essentially set the standards for overall biomarker laboratory operation, including assay validation, quality control, and quality assurance activities. The guidelines mandate regular proficiency testing for every

clinical assay, and CAP runs an extensive program providing proficiency testing samples for all commonly used clinical assays.

The FDA recently withdrew its enforcement discretion for certain LDTs that are marketed "direct to consumers," and previously[28] published a draft guidance proposing increased regulation of tests described as In Vitro Diagnostic Multivariate Index Assays. The recently released[27] draft guidance on Companion Diagnostics indicates the FDA's intent to ensure the availability of safe and effective tests when results from those tests are essential for the safe and effective use of a corresponding therapeutic product (drug, biologic, or device).

Systems for Level of Evidence

Ideally, clinical utility of a marker should be determined in a prospective clinical trial, as is required for new drugs. In fact, trial designs to determine clinical utility of markers have been described and several are underway.[29,30] One way to streamline the evaluation of both established and upcoming molecular tests, using the guidelines discussed earlier to evaluate the quality of studies, is to assign a well-defined level of evidence to each test, using standards of analytic and clinical validity and clinical utility. Levels-of-evidence standards for assessing tumor markers have been published, and provide a common comparison that can be used across all types of molecular testing. Two in particular, the Tumor Marker Utility Grading System (TMUGS[31]; Table 1) and the levels of evidence standards for using archived tissue[32] (Table 2), have been proposed to provide a common language to improve communication regarding assay development and validation.

Table 1 Tumor Marker Utility Grading System Levels of Evidence

Level	Definition
I	Prospective, marker primary objective Well-powered or meta-analysis
II	Prospective, marker the secondary objective
III	Retrospective, outcomes, multivariate analysis (most currently published marker studies are level of evidence III)
IV	Retrospective, outcomes, univariate analysis
V	Retrospective, correlation with other marker, no outcomes

Adapted from Hayes DF, Bast RC, Desch CE, et al. Tumor marker utility grading system: a framework to evaluate clinical utility of tumor markers. J Natl Cancer Inst 1996;88:1464; with permission.

The authors of TMUGS proposed an updated annotation for levels of evidence that combines both Tables 1 and 2, while acknowledging the importance of rigorous analysis of archived specimens from definitive clinical studies (Table 3).[32] The authors appropriately point out that biomarker development can challenge the semantic distinction between prospective and retrospective studies, and the critical element to biomarker validation is the robust statistical design of the study and definition of the biomarker parameters before application to the samples being used for validation. Although prospective collection of these samples in the setting of a clinical trial with objectives focused on the validation of the biomarker will remain the best means to minimize bias and obtain the highest level of evidence in support of a biomarker, robust analysis of archived specimens can also provide strong validation data. An example of the latter is the association of *KRAS* mutations and resistance to antibodies targeting epidermal growth factor receptors (EGFRs).[33,34] Thus, this revised levels of evidence system combines the original TMUGS system in Table 1 with the clinical trial design classification from Table 2 to create a system that can be used to classify tumor markers and also suggest what supportive validation studies are required for each level.

Although the evolution of systems with which to classify levels of evidence for biomarkers is likely to continue, the task force members believe that these level-of-evidence systems provide an appropriate initial mechanism of assigning levels of evidence for new assays that is consistent with how NCCN approaches the level of evidence and consensus for cancer therapies (Table 4). In the following sections that focus on specific diseases, the task force used the revised system proposed by Simon et al.[32] (Table 3) to annotate the level of evidence supporting each tumor marker, and has noted the NCCN category of evidence and consensus for the markers included within the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines; see Tables 5–10).

Assays Currently Used in Cancer Care

For each disease considered by the task force, including glioma, breast cancer, colon cancer, lung cancer, prostate cancer, and acute myelogenous

Table 2 Use of Archived Tissues to Determine Clinical Validity of Tumor Markers

Category Trial Design	A Prospective	B Prospective Using Archived Samples	C Prospective/ Observational	D Retrospective/ Observational
Clinical trial	PCT designed to address tumor marker	Prospective trial not designed to address tumor marker, but design accommodates tumor marker utility	Prospective observational registry, treatment and follow-up not dictated	No prospective aspect to study
Patients and patient data	Prospectively enrolled, treated, and followed in PRCT	Prospectively enrolled, treated, and followed up in clinical trial and, especially if a predictive utility is considered, a PRCT addressing the treatment of interest	Prospectively enrolled in registry, but treatment and follow-up standard of care	No prospective stipulation of treatment or follow-up; patient data collected through retrospective chart review
Specimen collection, processing, and archival	Specimens collected, processed, and assayed for specific marker in real time	Specimens collected, processed, and archived prospectively using generic SOPs; assayed after trial completion	Specimens collected, processed, and archived prospectively using generic SOPs Assayed after trial completion	Specimens collected, processed, and archived with no prospective SOPs
Statistical design and analysis	Study powered to address tumor marker question	Study powered to address therapeutic question and underpowered to address tumor marker question Focused analysis plan for marker question developed before performing assays	Study not prospectively powered at all; retrospective study design confounded by selection of specimens for study Focused analysis plan for marker question developed before performing assays	Study not prospectively powered at all; retrospective study design confounded by selection of specimens for study No focused analysis plan for marker question developed before performing assays
Validation	Result unlikely to be play of chance Although preferred, validation not required	Result more likely to be play of chance than A, but less likely than C Requires one or more validation studies	Result very likely to be play of chance Requires subsequent validation studies	Result very likely to be play of chance. Requires subsequent validation studies

Abbreviations: PCT, prospective controlled trial; PRCT, prospective, randomized controlled trial; SOP, standard operating procedure.

Adapted from Simon RM, Paik S, Hayes DF. Use of archived specimens in evaluation of prognostic and predictive biomarkers. J Natl Cancer Inst 2009;101:1449; with permission.

leukemia, tabular data were assembled on markers that are currently included in the NCCN Guidelines (available at www.NCCN.org), and new markers currently under investigation. For all listed biomarkers, NCCN categories of evidence and consensus were given if available, and combined levels of evidence calculations (Tables 1–3) were made and are included with references to illustrate the hurdles that prevent some new tests from being widely included in practice guidelines. The hope is that a critical examination of the published evidence on each of these tests will show why some, which may have been widely presented at meetings or advertised in public venues, do not achieve thresholds needed for broad adoption into clinical practice.

Molecular Testing in Glioma

Table 5 summarizes current molecular biomarkers in glioma.

1p/19q Deletion

The current NCCN Guidelines for Central Nervous System Cancers specify a single molecular test to help determine prognosis and select chemotherapy

Table 3 Revised Determination of Levels of Evidence Using Elements of Tumor Marker Studies*

Level of Evidence	Category From Table 2	Validation Studies Available
I	A	None required
I	B	One or more with consistent results
II	B	None or inconsistent results
II	C	2 or more with consistent results
III	C	None or 1 with consistent results or inconsistent results
IV–V	D	NA†

*Levels of evidence revised from those originally proposed in Tables 1 and 2.[31]
†Not applicable (NA) because level of evidence IV and V studies will never be satisfactory for determination of medical utility.
From Simon RM, Paik S, Hayes DF. Use of archived specimens in evaluation of prognostic and predictive biomarkers. J Natl Cancer Inst 2009;101:1450; with permission.

for certain types of central nervous system tumors. Testing for the 1p/19q codeletion, or unbalanced translocation, is recommended for cases of suspected oligodendroglioma. The test is also recommended to distinguish anaplastic oligodendroglioma from anaplastic astrocytomas and glioblastomas (to view the most recent version of these guidelines, visit the NCCN Web site at www.NCCN.org).[35]

Oligodendroglioma displays a loss of 1p/19q and is more sensitive to chemotherapy and radiotherapy than are astrocytic tumors. Presence of the translocation is a favorable prognostic factor, and has an NCCN category 1 designation, indicating that high-level evidence, either from randomized controlled clinical trials or meta-analyses, supports using this test.[36–42] Detection methods, not specified within the guideline, include polymerase chain reaction (PCR)–based testing for loss of heterozygosity, FISH, array comparative genomic hybridization, and multiplex ligation-dependant probe amplification (MPLA).[42–46] A practical method of FISH testing for 1p/19q deletion was recently published[47] and the authors note that no other consensus guidelines or protocols exist for 1p/19q testing using other technologies. This test is considered to have level IA evidence (Table 3).

Table 4 NCCN Categories of Evidence and Consensus

Category 1: Based upon high-level evidence, there is uniform NCCN consensus that the intervention is appropriate.

Category 2A: Based upon lower-level evidence, there is uniform NCCN consensus that the intervention is appropriate.

Category 2B: Based upon lower-level evidence, there is NCCN consensus that the intervention is appropriate.

Category 3: Based upon any level of evidence, there is major NCCN disagreement that the intervention is appropriate.

IDH1/IDH2 Mutation

Other molecular tests are rapidly gaining attention in central nervous system cancers and, with additional clinical data and validation, will most likely be considered for inclusion in upcoming treatment guidelines. One of these is mutation in the cytosolic isocitrate dehydrogenase gene (*IDH1*) or the mitochondrial version of the same gene (*IDH2*). IDH1 is involved in the metabolic conversion of isocitrate to alpha-ketoglutarate, which reduces NADP to NADPH. In glioma, mutations in this gene were discovered through large-scale sequencing of tumor DNA samples.[48] *IDH1* mutations were present in 12% of glioblastoma samples in this study. Later retrospective analyses of 271 clinical trial samples of low-grade glioma showed that mutated *IDH1* may be both prognostic and predictive, because it is associated with longer survival times and better response to temozolomide.[49,50] Another study of astrocytoma showed an association with improved survival but not with response to temozolomide.[51]

Among WHO grade II and III gliomas, 50% to 80% have mutated *IDH1*, whereas 5% to 10% of WHO grade IV gliomas bear a mutated *IDH1*. Roughly 90% of IDH1 mutated proteins can be detected using immunohistochemistry with a monoclonal antibody that detects p.R132H, the most common *IDH1* mutation.[52,53] A recommendation for optimal testing for this mutation might be to first perform immunohistochemical testing with this detecting antibody, then to follow up with DNA sequencing only when the results from immunohistochemistry are negative or equivocal. Further clarification of the correlation of chemotherapy response and *IDH1* mutation will be needed for this test to receive broad clinical use. At this point, lack of *IDH1* mutation could not be considered strong enough evidence to alter

therapy.[51,54] However, one study showed that *IDH* wild-type grade III tumors (usually treated with radiotherapy alone) are associated with a prognosis similar to grade IV tumors (treated with concurrent chemotherapy and radiotherapy).[55] If validated with further studies, *IDH* testing could therefore have an impact on patient management. Because the presence of an *IDH* mutation is considered tumor-specific, it has a role as a diagnostic marker when morphologic features are inconclusive and a nonneoplastic/reactive condition is possible. Overall, this marker is currently considered primarily prognostic/diagnostic, with the possibility that more data will indicate whether mutation status can be considered in recommending treatment. Studies showing the association have come from well-designed retrospective analyses, and evidence for the test is considered to be level IIB.

BRAF Fusion Protein

Alterations in the signaling-associated kinase BRAF have been noted in many tumors. These often occur as point mutants which lead to a constitutively active enzyme, BRAF c.1799T>A (p.V600E).[56–58] Most pilocytic astrocytomas have been shown to express a fusion protein of KIAA1549:BRAF, which is constitutively active.[59] Because pilocytic astrocytoma can be cured through surgery alone, testing for the presence of the fusion protein might be useful to aid in the diagnosis of pilocytic astrocytoma when histologic features are inconclusive and difficult to distinguish from a diffuse astrocytoma. Therefore the combination of *IDH* mutation testing (common in diffuse astrocytoma) with *BRAF* testing represents a rational diagnostic panel for cases with indeterminate histologic features. Testing methodologies used in research studies include long-distance inverse PCR to map breakpoints on the genomic level, 5′ rapid amplification of cDNA ends (RACE) to detect gene fusion, and FISH using probes that map to the KIAA1549 sequence and to the *BRAF* sequence.[50] Implementation of the FISH assay will probably be the most straightforward for clinical testing, as this can be performed on FFPE samples, but lack of commercially available probe sets and assay standardiza-

Table 5 Current Molecular Biomarkers in Glioma

Biomarker	Molecular Compartment	Purpose	Analytic Validity Demonstrated	Level of Evidence	NCCN Category of Evidence	References
Markers With Accepted Clinical Utility						
1p/19q codeletion (unbalanced translocation)	Tumor DNA	Diagnostic (oligodendroglioma)	FISH, aCGH, LOH, MPLA	IA	1	Smith et al.[46]
IDH mutation (*IDH1*) c. 395 G>A p.R132H (*IDH2*)	Tumor DNA, tumor protein	Positive is favorably prognostic; also a diagnostic marker	IHC, DNA sequencing	IIB		Houillier et al.[49] Dubbink et al.[51]
MGMT methylation	Tumor DNA	Prognostic, predictive (benefit for chemotherapy), pharmacodynamic (pseudorecurrence)	MS-PCR, MS-pyrosequencing, MS-MPLA	IIB		Hegi et al.[61] Gilbert et al.[215]
Markers With Emerging Evidence						
BRAF fusion (pilocytic astrocytoma)	Tumor DNA	Diagnostic (pilocytic astrocytoma)	LDI-PCR, 5′ RACE, FISH	IIB		Jeuken and Wesseling.[216] Jones et al.[59]
CIMP (CpG island methylator phenotype)	Tumor DNA	Positive is favorably prognostic	Gene expression microarray, pyrosequencing	IIB		Noushmehr et al.[65] Gilbert et al.[215]

Abbreviations: aCGH, array comparative genomic hybridization; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; LDI-PCR, long-distance inverse polymerase chain reaction; LOH, loss of heterozygosity; MPLA, multiplex ligation-dependant probe amplification; MS, methylation-specific; MS-MPLA, methylation-specific multiplex ligation-dependant probe amplification; MS-PCR, methylation-specific polymerase chain reaction; RACE, rapid amplification of cDNA ends.

tion to date still must be overcome for this test to be broadly implemented.[50] The association has been shown through well-designed retrospective analyses, and evidence for the test is considered to be level IIB.

O-6-Methylguanine-DNA-Methyltransferase Promoter Methylation

Activity of DNA repair enzyme O-6-methylguanine-DNA-methyltransferase (MGMT) is associated with resistance to chemotherapeutic alkylating agents. Silencing of the *MGMT* promoter through methylation has been shown to be associated with better outcomes (overall survival, progression-free survival) in a variety of glioma subtypes and to partially predict response to alkylating agents.[60–63] *MGMT* promoter methylation has also been shown to be positively associated with therapeutic pseudo-regression. One barrier to use of *MGMT* as a prognostic marker, or as a marker for pseudo- versus true regression, is that the methodology for assessing *MGMT* is not yet standardized.[50,64] Measurement of *MGMT* protein levels using immunohistochemical testing does not correlate with extent of *MGMT* promoter methylation as measured using methylation-specific PCR. Promoter methylation itself can be measured using a variety of technologies, including methylation-specific PCR, methylation-specific pyrosequencing, and methylation-specific MPLA. Each of these assays has benefits and drawbacks in terms of reproducibility, standardization of cutoffs, and ease of use, and which will emerge as the best for use in a clinical setting remains to be seen.[50,64] The association has been shown through well-designed retrospective analyses, and evidence for the test is considered to be level IIB.

Glioma-CpG Island Methylator Phenotype

A more-extensive methylation analysis of glioblastoma tumors showed a glioma-CpG island methylator phenotype (G-CIMP) similar to that observed in colorectal cancer.[65,66] The G-CIMP phenotype is colocalized with the *IDH1* mutation, and is found predominately in lower-grade tumors from younger patients with longer survival times. Promoter methylation is observed in a subset of loci, defining a set of glioma tumors. Methods involved assessing promoter methylation and gene expression for a training set of tumors, and assembling a list of genes that were both transcriptionally silenced and methylated. These results, though intriguing, will require further analytic validation, assay development, and especially demonstration that the G-CIMP phenotype is associated with greater or lesser sensitivity to treatment regimen. The association has been shown through well-designed retrospective analyses, and evidence for the test is considered to be level IIB (Tables 1–3).

Overall, use of central nervous system tumor biomarkers is in its infancy, with several new and exciting candidates that have the potential to change how tumors are evaluated and may help determine treatment. The challenge in this area will be to ensure that clinical recommendations encompass validated assays, including specific recommendations of methodology and scoring, and include comparisons of technologies used to assess these biomarkers.

Molecular Testing in Breast Cancer

Table 6 summarizes current molecular biomarkers in breast cancer.

ER-α(ESR1)/PgR(PR)

The measurement of estrogen receptor expression is prognostic[67] and predictive for response to *ER-α(ESR1)*-modulating agents.[68] The prognostic and treatment predictive use of progesterone receptor expression is less clear.[69–71] Testing for *ER-α(ESR1)* in ductal carcinoma in situ and *ER-α(ESR1)/PgR(PR)* expression in breast tumors is a necessary component of the initial workup for these diseases.[72] Guidelines for immunohistochemical testing in breast cancer have been published by ASCO, CAP, and NCCN, with detailed information available on all aspects of testing, including tissue handling and assay validation.[73–76] The NCCN category of evidence and consensus for use of this test is 2A. Using the TMUGS and Simon et al.[32] criteria for assessing marker validation gives this test a rating of IB as a predictive test (Table 3).

HER2(ERBB2)

Expression of *HER2(ERBB2)* in breast tumors is also both a prognostic and predictive marker. *HER2(ERBB2)*-positive tumors respond to trastuzumab, a monoclonal antibody directed against the extracellular domain of the *HER2(ERBB2)* protein, and lapatinib, a tyrosine kinase inhibitor, versus *HER2(ERBB2)*-negative tumors which do not seem to respond. Clinical trial results have shown that adjuvant trastuzumab reduces recurrence and mortality by approximately 50% and 30%, respec-

Table 6 Current Molecular Biomarkers in Breast Cancer

Biomarker	Molecular Compartment	Purpose	Analytic Validity Demonstrated	Level of Evidence	NCCN Category of Evidence	References
Markers With Accepted Clinical Utility						
ER-α/PgR (ESR1/PR)	Tumor protein	Diagnostic prognostic (weak) predictive	IHC	Predictive: IB	2A	Early Breast Cancer Trialists Collaborative Group[68] Hammond et al.[74] Hammond et al.[75] Allred et al.[73]
HER2(ERBB2)	Tumor protein	Diagnostic (classification) prognostic (favorable) predictive for anti-*HER2(ERBB2)* therapy	FISH, IHC	Predictive: IA	2A	Wolff et al.[78] Carlson et al.[77] Joensuu et al.[10] Piccart-Gebhart et al.[11] Romond et al.[12]
Oncotype Dx	Tumor RNA	Prognostic predictive	21-gene RT-PCR expression assay of FFPE samples with recurrence score as readout	Prognostic in ER+/node-negative patients: IB Predictive: IIA	Selection of treatment options based on Oncotype Dx score is category 2B, but use of the test in a defined subpopulation of patients is 2A	Paik et al.[80] Paik et al.[81] Albain et al.[79]
Markers With Emerging Evidence						
MammaPrint	Tumor RNA	Not clear	Multigene microarray expression assay using frozen tissue	IIB		Straver et al.[217] Knauer et al.[218]
CTCs	Circulating tumor cells	Monitor patients with metastatic disease	Cell surface staining and magnetic separation	IB		Budd et al.[219] Cristofanilli et al.[90,220] De Giorgi et al.[221]

Abbreviations: CTC, circulating tumor cell; ER, estrogen receptor; FFPE, formalin-fixed paraffin-embedded; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; RT-PCR, reverse transcriptase polymerase chain reaction.

tively, in *HER2(ERBB2)*-positive patients,[10–12] and inclusion of trastuzumab in the therapy regimen for *HER2(ERBB2)*-positive patients is given a category 1 recommendation in the NCCN Guidelines for Breast Cancer,[72] with a combined level of evidence score of 1A. Accurate testing for *HER2(ERBB2)* is thus critically important in developing treatment plans for breast cancer. NCCN and ASCO/CAP have both made extensive and detailed guidelines on *HER2(ERBB2)* testing for both FISH and immunohistochemistry,[77,78] and the NCCN Guidelines for Breast Cancer contain a section devoted to this issue[72] (available online, at www.NCCN.org [BINV-A]), which details specific thresholds and confirmation of positive and negative results.

Oncotype Dx

The Oncotype Dx (Genomic Health, Inc., Redwood City, CA) test is a 21-gene reverse transcriptase PCR (RT-PCR) assay that is performed at a single site as a laboratory-developed test, using FFPE breast tumor samples as a source of RNA. Gene expression levels are translated into a recurrence score.[79–81] The current NCCN Guidelines recommend the 21-gene recurrence score be considered for determining prognosis in node-negative, *ER-α(ESR1)*-positive breast tumors. Patients with a low recurrence score have such a favorable prognosis that even if chemotherapy were beneficial, so few patients would benefit that the risks outweigh the benefits. The NCCN category of evidence and consensus for using Oncotype DX to determine whether a patient receives chemotherapy is 2B.

This recommendation is echoed by ASCO in its 2007 recommendations for use of tumor markers in breast cancer.[82] In its review of tumor gene expression profiling[72,82,83] in breast cancer, the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) working group found insufficient evidence to recommend Onco*type* Dx but noted that encouraging indirect evidence exists for clinical utility of the test.[84]

Provocative but still hypothesis-generating studies have suggested that in addition to being prognostic in women with *ER*-α(*ESR1*)-positive, node-negative tumors, the 21-gene recurrence score may also be predictive of the relative benefits of chemotherapy.[79,81] These studies have suggested that tumors with low recurrence scores are relatively resistant to chemotherapy, whereas those with higher recurrence scores are increasingly sensitive, independent of the prognostic effect of the assay.

The recently completed TAILORx trial will determine the benefit of chemotherapy in node-negative women with intermediate risk scores, and will further determine the prognostic role of the assay in patients with a low recurrence score.[85] The ongoing S01007/RxPONDER trial will prospectively determine the benefits of adjuvant chemotherapy in node-positive, *ER*-α(*ESR1*)-positive patients.[86] As trial results become available, and if they continue to show a demarcation in value for chemotherapy, this assay is expected to become more widely recommended. Currently, the level of evidence score for Onco*type* Dx as a prognostic test is IB, whereas as a predictive test, it is IIA.

MammaPrint

The MammaPrint 70-gene microarray assay (Agendia, Irvine, CA) is the only multigene breast cancer assay that has received FDA clearance.[87,88] However, 3 major groups that have considered it have not recommended its use.[72,82,84] It is useful to remember that the FDA does not make considerations of clinical utility, only of analytic validity and clinical validity in its determinations of clearance. One barrier to the use of MammaPrint is the requirement of fresh-frozen tissue samples, rather than the more universally available FFPE tumor samples. In addition, currently completed studies do not assess the ability of MammaPrint to predict benefit from treatment. An ongoing trial (MINDACT) will compare the effectiveness of MammaPrint results to typical clinical evaluation in the prediction of survival at 15 years.[89] Until trial results are available, MammaPrint has a combined level of evidence of IIB as a prognostic test.

Circulating Tumor Cells

The CellSearch circulating tumor cell (CTC) detection system (Veridex, LLC, Raritan, NJ) has been cleared for use in metastatic breast cancer by the FDA. However, this test is not recommended by the ASCO breast cancer tumor marker panel and is not included in the NCCN Guidelines for Breast Cancer.[72,82] The test uses an immunomagnetic system to isolate cells with the features of epithelial cells from the peripheral blood and provides an absolute count of CTCs per 7.5-mL blood sample. Presence of greater than 5 CTCs per 7.5-mL sample has been shown to be associated with shortened progression-free (2.7 vs. 7 months) and overall survivals (10.1 vs. > 18 months).[90,91] A randomized clinical trial, SWOG S0500, is designed to determine whether elevated CTC levels after initiation of treatment indicate that a change in chemotherapy regimen will be beneficial in the metastatic setting. Results of this trial will add to the clinical utility of this assay, and may allow it to be further considered by guidelines panels. Until those results are available, clinical validity for the determination of CTCs in breast cancer has a combined level of evidence score of IB.

Molecular Testing in Colon Cancer

Table 7 summarizes current molecular biomarkers in colon cancer.

KRAS Mutations

The RAS/RAF/MAPK and downstream pathways are activated in response to stimulation of the EGFR, and mutations to proteins in this pathway have been investigated for their role in modulating response to EGFR-targeting agents. Approximately 40% of colon cancers are positive for mutations in *KRAS*,[92–94] and colon cancers with *KRAS* mutations in codons 12 and 13 have been shown to be unresponsive to EGFR-targeting monoclonal antibody therapies, such as cetuximab or panitumumab.[33,95,96] The NCCN Guidelines for Colon Cancer recommend, with a category of 2A, the testing of all metastatic disease for the presence of mutations in *KRAS* (to view the most recent version of these guidelines, visit the NCCN Web site at www.NCCN.org).[97] An

ASCO provisional clinical opinion[98] also recommends *KRAS* mutation testing in metastatic colon cancers. No FDA-approved *KRAS* mutation test exists, and both publications mention the necessity of performing the test in a CLIA-certified facility. The ASCO Provisional Clinical Opinion notes that the test can be performed using either real-time PCR with mutation-specific primers, or sequencing. The NCCN Guidelines do not recommend any particular methodology. Testing for *KRAS* mutations can also be given a combined level of evidence of IB, owing to multiple randomized controlled trials of panitumumab or cetuximab in colon cancer, with evaluation of *KRAS* mutation status in archived samples.[33,34,96] Recent reports have shown that tumors harboring *KRAS* mutations at the c.38G>A (p.G13D) position may still be responsive to cetuximab, unlike other activating codon 12 and 13 mutations.[99,100] The NCCN Guidelines note that the use of EGFR-targeting agents in *KRAS* codon 13 mutant tumors is investigational, and is not recommended for routine clinical practice at this time.[97]

BRAF c.1799T>A (p.V600E) Mutation

The *BRAF* c.1799T>A (p.V600E) mutation renders the kinase domain of the protein constitutively active, and is found in 10% to 20% of colon tumors.[56–58,101,102] RAF is downstream of EGFR, and a constitutively activated RAF enzyme might therefore circumvent EGFR-directed therapy. Retrospective studies have shown that mutated *BRAF* is associated with decreased progression-free and overall survivals in patients undergoing anti-EGFR–containing therapy,[103–105] although interpretation of this association is confounded by its strong negative prognostic implications. Randomized data suggest, however, that patients with a *BRAF* mutation may benefit from EGFR-directed therapy in the first-line treatment setting, despite the unfavorable prognosis conferred by this mutation.[95] The NCCN Guidelines for Colon Cancer (available at www.NCCN.org) recommend that *BRAF* testing be considered in the metastatic setting if the *KRAS* gene is found to be unmutated. This is given a category of evidence of 2A.[97] Combined level of evidence for the use of *BRAF* testing for prognostic information is IB, owing to multiple retrospective analyses of randomized clinical trial samples.[58,95,103,105–107]

Mutations of *KRAS* and *BRAF* almost never coexist in the same tumor.[58] Testing for *BRAF* is recommended to be performed in a CLIA-certified laboratory, and is usually accomplished using Sanger sequencing, which can be performed with FFPE samples containing at least 40% neoplastic cellularity (~20% mutant alleles); detection limit can be 5% to 10% mutant alleles or lower in pyrosequencing and real-time PCR with or without melting curve analysis.[108–110] Testing for *BRAF* mutation in colon cancer is primarily through laboratory-developed tests, because, other than the test recently approved for melanoma, no FDA-cleared *BRAF* mutation tests are currently available.

Microsatellite Instability

Defects in mismatch repair (MMR) can be detected using either immunohistochemistry for the absence of MLH1, MSH2, MSH6, and/or PMS2 protein in tumor tissues, or through a comparative PCR analysis of tumor and normal patient DNA for microsatellite instability (MSI). Hereditary syndromes with a predisposition to the development of colon cancer, such as Lynch syndrome, have been shown to contain germline mutations in one of the MMR genes, whereas most sporadic MSI-high colon tumors show MLH1 promoter hypermethylation and epigenetic silencing.[102,109,111,112] In general, MSI-high colon cancers are associated with good prognosis, and patients with MSI-high stage II colon cancers do not seem to benefit from adjuvant single-agent 5-FU chemotherapy.[113–115]

The NCCN Guidelines currently recommend testing for MMR proteins in all patients younger than 50 years and/or who meet the revised Bethesda guidelines or Amsterdam criteria because of the increased possibility of Lynch syndrome in this cohort, and with a level 2A category of evidence. MMR/MSI testing is also recommended for patients with stage II tumors if single-agent fluoropyrimidine adjuvant therapy is considered, given evidence for lack of benefit and possibly worse outcomes in this population (NCCN Guideline, category 2A).[97] Use of MSI/MMR testing as a screen for Lynch syndrome has a combined level of evidence score of IB,[116–119] as does use of this test for prognostic purposes,[106,120,121] owing to the results of multiple studies, including both randomized clinical trials and meta-analyses. The ASCO recommendation for use of tumor markers in colorectal cancer does not endorse routine testing for MSI or MMR in all patients,[122] but this recommendation may be reconsidered when the position statement is updated. No FDA-cleared tests are available for MSI testing.

Table 7 Current Molecular Biomarkers in Colon Cancer (Cont. on facing page)

Biomarker	Molecular Compartment	Purpose	Analytic Validity Demonstrated	Levels of Evidence	NCCN Category of Evidence	References
Markers With Accepted Clinical Utility						
KRAS mutations [except c.38G>A (p.G13D)]*	Tumor DNA	Predictive (negative for anti-EGFR therapy); negatively prognostic in several first-line randomized studies	Multiple methods: PCR, multiplex assays, direct sequencing	Predictive: IB Prognostic: IIB	2A	Amado et al.[33] Karapetis et al.[34] Van Cutsem et al.[96] Tol et al.[222] Douillard et al.[223]
MSI and/or MMR protein loss	Tumor DNA for MSI testing with PCR; tumor IHC for MMR proteins	Screening (Lynch syndrome) Prognostic (recurrence, overall survival) Predictive (lack of benefit, possibly worse outcome with adjuvant single-agent fluoropyrimidine therapy)	PCR, IHC	Screening: IB Prognostic: IB Predictive: IIB	2A	Screening for Lynch: Hampel et al.[116] Umar et al.[118] Prognostic: Hutchins et al.[120] Popat et al.[121] Roth et al.[106] Predictive: Des Guetz et al.[113] Guastadisegni et al.[114] Sargent et al.[115]
CEACAM5 (CEA)	Patient serum	Surveillance	Immunoassay	IIC	2A	Wanebo et al.[126] Wang et al.[127] Locker et al.[122]
BRAF c.1799T>A (p.V600E) mutation	Tumor DNA	Prognostic (strong negative prognostic marker) Predictive (negative for anti-EGFR therapy)	Multiple methods: PCR, multiplex assays, direct sequencing	Prognostic: IB Predictive: IIIC	2A	Bokemeyer et al.[95] De Roock et al.[103] Ogino et al.[58] Roth et al.[106] Samowitz et al.[107] Tol et al.[105]

Abbreviations: CTC, circulating tumor cell; EGFR, epidermal growth factor receptor; FFPE, formalin-fixed paraffin-embedded; H&E, hematoxylin and eosin; IHC, immunohistochemistry; MMR, mismatch repair; MSI, microsatellite instability; PCR, polymerase chain reaction; RT-PCR, reverse transcriptase polymerase chain reaction.

*All testing for *KRAS* mutation is presumed to have the same analytic validity, and will probably be performed simultaneously. However, presence of a c.38G>A (p.G13D) mutation does not yet meet the standard of clinical utility needed to drive a treatment decision.

Immunohistochemistry testing for MMR proteins has been shown to be a reliable method for detecting defects in the DNA MMR system, and is generally concordant with MSI testing with PCR.[117,123] Germline mutation (plus a second hit) or silencing through promoter methylation both lead to an absence of functional protein, detectable through immunohistochemistry in most instances. The presence of a *BRAF* mutation in addition to the finding of MMR deficiency and/or MSI-high indicates a sporadic acquisition and may be used to distinguish from the hereditary Lynch syndrome.[124,125] Reflexive testing of all newly diagnosed colorectal cancers for the presence of MMR deficiency and/or MSI, along with *BRAF* mutational status if MMR deficiency and/or MSI-high status are detected, may be recommended in the future as part of standard pathologic review pending guideline updates from CAP.

CEACAM5 (CEA)

Testing for carcinoembryonic antigen–related cell adhesion molecule 5, or CEACAM5 (so-called CEA), which is released from tumor cells into patient serum, is recommended by the NCCN Guidelines for Colon Cancer and ASCO.[97,122] Testing is recommended on di-

Table 7 Current Molecular Biomarkers in Colon Cancer (Cont.)

Biomarker	Molecular Compartment	Purpose	Analytic Validity Demonstrated	Levels of Evidence	NCCN Category of Evidence	References
Markers With Emerging Evidence						
KRAS c.38G>A (p.G13D) mutation*	Tumor DNA	Predictive (negative for anti-EGFR therapy)	Multiple methods: PCR, multiplex assays, direct sequencing	IIB		De Roock et al.[99] Tejpar et al.[100]
CTCs	Whole blood	Prognostic	Cell surface staining and magnetic separation	IB		Cohen et al.[128] Rahbari et al.[129] Tol et al.[130]
ColoPrint	Tumor mRNA	Prognostic in stage II	Microarray	IIC		Salazar et al.[132] Rosenberg et al.[131]
Oncotype Dx Colon	Tumor mRNA	Prognostic in stage II	RT-PCR expression assay of FFPE samples with recurrence score as readout	IB		Kerr et al.[224] Venook et al.[225]
CIMP (CpG island methylator phenotype) status	Tumor DNA	Prognostic	No	IIIC		Ogino et al.[58] Dahlin et al.[133] Samowitz et al.[102] Barault et al.[134]
LINE-1 hypomethylation	Tumor DNA	Prognostic	Yes	IIIC		Ogino et al.[135] Ahn et al.[136]
Immune cells	H&E or IHC	Prognostic	No	IIIC		Ogino et al.[137] Dahlin et al.[138] Nosho et al.[139] Ogino et al.[137] Galon et al.[141] Mlecnik et al.[142]

Abbreviations: CTC, circulating tumor cell; EGFR, epidermal growth factor receptor; FFPE, formalin-fixed paraffin-embedded; H&E, hematoxylin and eosin; IHC, immunohistochemistry; MMR, mismatch repair; MSI, microsatellite instability; PCR, polymerase chain reaction; RT-PCR, reverse transcriptase polymerase chain reaction.
*All testing for *KRAS* mutation is presumed to have the same analytic validity, and will probably be performed simultaneously. However, presence of a c.38G>A (p.G13D) mutation does not yet meet the standard of clinical utility needed to drive a treatment decision.

agnosis of invasive colon cancer, which can be used as a baseline for monitoring. CEACAM5 (CEA) is then tested regularly, and increasing levels are one indication of recurrent disease or metastasis. FDA-approved immunoassays are available for CEACAM5 (CEA) monitoring. Use of this test is a category 2A recommendation in the NCCN Guidelines, and the combined level of evidence score is IIC, because most studies on this marker have been observational.[97,122,126,127] Neither guideline recommends CEACAM5 (CEA) as a screening or diagnostic tool.

CTCs

Detection of CTCs in patients with advanced colorectal cancer is a negative prognostic factor, correlating with reduced progression-free and overall survivals.[128–130] The clinical utility of this test is unclear. CTC testing is not included in the NCCN Guidelines for Colon Cancer (to view the most recent version of these guidelines, visit the NCCN Web site at www.NCCN.org). As a prognostic tool, evidence for the clinical validity of CTC testing is level IB.

ColoPrint

The ColoPrint assay uses oligonucleotide microarray technology to assign a recurrence score based on expression of 18 genes, and currently requires fresh frozen tumor tissue as a source of RNA. Higher recurrence scores are associated with shorter time to progression and shorter overall survival in patients with stage I through III colon and rectal cancers, but

are not predictive of chemotherapy benefit.[131,132] The test can be considered to have developed clinical validity, with a IIC level of evidence, but does not have proven clinical utility at this time. It is not included in the NCCN Guidelines for Colon Cancer (available at www.NCCN.org).

Oncotype Dx Colon Assay

The Oncotype Dx Colon assay is a 12-gene RT-PCR expression assay that can be performed on FFPE tumor samples. This is also a prognostic test, with higher recurrence scores associated with shorter disease-free and overall survivals. The Oncotype Dx colon recurrence score has established clinical validity in its association with recurrence risk in patients with stage II colon cancer in 2 independent randomized validation studies, with a level of evidence of IB, but its clinical utility has not yet been established. It is not included in the NCCN Guidelines for Colon Cancer (available at www.NCCN.org).

Other Biomarkers

Other tests, including CIMP status,[58,102,133,134] LINE-1 (global DNA) hypomethylation,[135,136] and presence of infiltrating immune cells within colon tumors[137–142] are also under current investigation for their use in guiding colon cancer treatment. These tests will require further analytic and clinical validation, and demonstration of clinical utility before they should be considered for use in general practice.

Molecular Testing in Non–Small Cell Lung Cancer

Table 8 summarizes current molecular biomarkers in non–small cell lung cancer (NSCLC).

EGFR Mutation

The EGFR tyrosine kinase is expressed in most lung cancers, and in 2004, somatic kinase domain mutations that led to a constitutively activated EGFR were shown to be associated with high sensitivity to the small-molecule EGFR kinase inhibitors gefitinib and erlotinib.[143–145] The most common mutations are exon 19 deletions (LREA deletion) and exon 21 point mutations (most commonly L858R),[146] which together account for 85% to 90% of *EGFR* mutations in lung cancer. *EGFR* mutations are found more often in women, never-smokers, and East Asians.[147] Because the presence of a mutation confers sensitivity to tyrosine kinase inhibition, and nonmutated *EGFR* tumors do not respond to EGFR inhibition, the NCCN Guidelines for NSCLC recommend testing recurrent or metastatic adenocarcinoma of the lung for *EGFR* mutation, with a category 1 recommendation and a combined level of evidence score of IA owing to multiple randomized clinical trials (to view the most recent version of these guidelines, visit the NCCN Web site at www.NCCN.org).[148] In early 2011, ASCO published a preliminary clinical opinion,[149] indicating that analysis of clinical trial data suggest a benefit for EGFR testing. A variety of methods are available for testing for *EGFR* mutations,[150] and the current guidelines do not recommend a particular test, noting only that mutation testing is preferable to FISH assessment of EGFR copy number or immunohistochemistry to assess EGFR protein levels.[151–153] A collaborative effort between CAP, the Association for Molecular Pathology (AMP), and the International Association for the Study of Lung Cancer (IASLC) is scheduled to produce detailed guidelines for EGFR and ALK testing in lung cancer, which should be a valuable resource for guideline developers and practitioners.

KRAS Mutation

Constitutively activating *KRAS* mutations are detected in approximately 20% of patients with NSCLC, mainly in those with adenocarcinoma and in smokers. Several studies analyzing both *EGFR* and *KRAS* mutations have shown that these mutations are present in mutually exclusive populations,[154–156] and that the presence of a *KRAS* mutation is a negative prognostic factor in the absence of treatment. KRAS is a downstream component of the EGFR signaling pathway, and activating mutations in this protein might therefore be expected to be unaffected by EGFR inhibition. *KRAS* mutant tumors have been shown to be resistant to gefitinib or erlotinib therapy.[157–159] However, the NCCN Guidelines for NSCLC (available at www.NCCN.org) currently do not recommend *KRAS* testing for any particular patient population because the impact on progression-free or overall survival is unclear,[148] and the combined level of evidence score for *KRAS* testing is IIB. Notably, no one has performed a study randomizing patients with *KRAS* mutant tumors to erlotinib versus chemotherapy. However, a tumor that has a *KRAS* mutation is highly likely to be *EGFR* wild-type, and therefore the *EGFR* wild-type status can be used to inter-

Table 8 Current Molecular Biomarkers in Non–Small Cell Lung Cancer

Biomarker	Molecular Compartment	Purpose	Analytic Validity Demonstrated	Level of Evidence	NCCN Category of Evidence	References
Markers With Accepted Clinical Utility						
EGFR mutation	Tumor DNA	Predictive	Multiple methods: PCR, multiplex assays, direct sequencing	IA	1	Mok et al.[226] Yang et al.[227] Fukuoka et al.[228] Lee et al.[229] Maemondo et al.[230] Mitsudomi et al.[231]
ALK gene fusion	Tumor DNA	Predictive (crizotinib)	FISH	IIB	2A	Kwak et al.[160]
Markers With Emerging Evidence						
KRAS mutation	Tumor DNA	Predictive	Multiple methods: PCR, multiplex assays, direct sequencing	IIB		Pao et al.[158] Linardou et al.[232] Mao et al.[233]
ERCC1	Tumor protein	Predictive (poor response to platinum chemotherapy)	IHC, FISH	IIB		Simon et al.[167] Olaussen et al.[166]

Abbreviations: EGFR, epidermal growth factor receptor; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; PCR, polymerase chain reaction.

pret use of EGFR tyrosine kinase inhibitors. Furthermore, *KRAS* mutations define a distinct subset of lung cancer that may be treated with different therapies in the future.

ALK Gene Fusion

A chromosomal rearrangement resulting in a fusion protein involving the ALK tyrosine kinase is found in 2% to 7% of patients with NSCLC.[160,161] Most of the time, the 5′ fusion partner is EML4, but other 5′ partners, such as KIF5B and TFG, are rarely identified. Patients with *ALK* translocations are similar to those with *EGFR* mutations in that they are more often never-smokers with adenocarcinoma; however, similar to *EGFR* mutations, *ALK* fusions can be found in all types of lung adenocarcinoma, regardless of clinical features. For this group of patients, ALK inhibition represents a valuable therapeutic tool. The FDA recently approved crizotinib, a small molecule inhibitor of ALK,[160,162,163] together with a companion diagnostic test for the translocation.[164] FISH and immunohistochemistry have both been used to detect *ALK* fusion products, and some recommend confirming mutation status using both assays,[161] but testing for *ALK* fusion using FISH was an enrollment criterion for the crizotinib trials, and this is the only FDA-approved *ALK* test available at this time.

Because *ALK* expression is lower in lung tumors than in anaplastic large cell lymphoma (which is also characterized by *ALK* gene fusions), a more sensitive immunohistochemistry test is needed[165] for use in lung tumors. Testing for *ALK* fusions is included in the most recent NCCN Guidelines for NSCLC with a category 2A designation,[148] and has a combined level of evidence of IIB.

ERCC1 Expression

ERCC1 is a component of the DNA excision repair pathway, and is required for the repair of DNA lesions introduced by ultraviolet light or formed by compounds such as cisplatin. High levels of ERCC1 expression in NSCLC, as measured with either RT-PCR or conventional immunohistochemistry, correlate with longer survival times in the absence of chemotherapy.[166,167] However, tumors with low expression of ERCC1 do derive benefit from adjuvant cisplatin-based chemotherapy, whereas those with high levels of ERCC1 expression do not. The test, or any particular methodology, is not currently recommended in the NCCN Guidelines, and can be considered to have achieved a IIB level of evidence.

Other Biomarkers

Several additional somatic mutations detected in lung cancer and molecular tests may enter clinical practice for NSCLC in the near future. These in-

clude mutations in *BRAF*, *HER2*(*ERBB2*), *AKT1*, *MAP2K1* (*MEK1*), and *PIK3CA*; gene amplifications in *MET*; or fusions involving the *ROS* tyrosine kinase. Each of these is present in a small percentage of patients with lung cancer (1%–5%), and will require further validation before being placed into general use to direct treatment.[168]

Rebiopsies

A serious limitation in lung cancer molecular testing is availability of tumor tissue. One recommendation to increase the amount of tissue available for testing is to use residual cells from a fine needle aspiration (FNA) to make a paraffin block. This can be done by rinsing the needle into a tissue block. Alternatively, during an FNA or CT-guided core needle biopsy, additional passes can be obtained through the tumor tissue. Patients could also undergo rebiopsy if molecular testing is needed. Notably, the ASCO guidelines also support efforts to increase the availability of tumor tissue for testing.[169]

Molecular Testing in Prostate Cancer

Table 9 summarizes current molecular biomarkers in prostate cancer.

Prostate-Specific Antigen (*KLK3*)

Prostate-specific antigen (PSA) is a peptidase (gene designation, *KLK3*) found in seminal plasma. Serum levels of this protein rise with the presence of prostate cancer and other conditions, such as benign prostatic hyperplasia or inflammation. Several large-scale screening trials in the United States and Europe[170–172] have shown a marginal, albeit significant, clinical outcome benefit (decreased deaths from prostate cancer) from use of the serum PSA(KLK3) test with digital rectal examination (DRE) in screening populations of healthy men for prostate cancer. Testing for PSA(KLK3) leads to earlier identification of small tumors, and an increased potential for overtreating tumors that may not be life-threatening. Guidelines for the appropriate use of PSA(KLK3) testing in prostate cancer screening continue to evolve (e.g., NCCN Guidelines for Prostate Cancer Early Detection and for Prostate Cancer; to view the most recent version of these guidelines, visit the NCCN Web site at www.NCCN.org). Changes in PSA(KLK3) level over time, or PSA(KLK3) velocity testing, may also be used to detect disease in patients who have low to intermediate risk of prostate cancer on initial testing,[173] and thus to detect potentially aggressive tumors while local treatment is still possible.

Serum PSA(KLK3) testing is also used to monitor prostate cancer after diagnosis, and consistently rising levels are used as one indicator of potential disease progression.[174] After definitive local treatment (e.g., radical prostatectomy or radiation therapy) for clinically localized prostate cancer, serum PSA(KLK3) levels generally drop to undetectable or very low levels. Rising levels of serum PSA(KLK3) in these patients indicates disease recurrence, and monitoring serum PSA(KLK3) levels in these men is of clinical utility.[175,176]

Table 9 Current Molecular Biomarkers in Prostate Cancer

Biomarker	Molecular Compartment	Purpose	Analytic Validity Demonstrated	Level of Evidence	NCCN Category of Evidence	References
Markers With Accepted Clinical Utility						
PSA(*KLK3*)	Serum protein	Diagnostic	Immunoassay	IA	2A	Schroder et al.[172] Hugosson et al.[171] Andriole et al.[170]
Markers With Emerging Evidence						
PCA3	Urine RNA	Diagnostic	RT-PCR	IB		Hessels and Schalken[178] Freedland and Partin[234] Chun et al.[177] Nogueira et al.[235]
CTCs	Whole blood	Prognostic	Immunomagnetic separation	IIA		de Bono et al.[236] Danila et al.[237] Scher et al.[182]

Abbreviations: CTC, circulating tumor cell; RT-PCR, reverse transcriptase polymerase chain reaction.

Although absolute thresholds remain a topic of discussion and controversy, use of PSA(KLK3) measurement in combination with DRE and Gleason score to diagnose prostate cancer is given a category 2A recommendation in the NCCN Guidelines for Prostate Cancer,[174] and can be given a combined level of evidence score of IA owing to long-term, prospectively designed, biomarker-focused clinical studies. Commercial tests for the detection of PSA(KLK3) have been cleared for use by the FDA.

PCA3

Although the use of serum PSA(KLK3) testing as a screening tool for prostate cancer is widespread, it has well-known limitations as an early-detection biomarker. Additional biomarkers that can improve on the sensitivity and specificity for early detection and monitoring of prostate cancer continue to be of great interest.

Several molecular alterations have been shown to be common in prostate cancer specimens, which are highly specific to the disease. The most well studied of these, with potential as clinical tools, are DNA methylation and overexpression of certain RNAs in the urine. One of these markers that has shown clinical promise is the PCA3 RNA test. PCA3 (DD3) is a noncoding RNA that can be detected in the urine after DRE, and which is highly expressed in prostate cancer compared with normal prostate.[177,178] A test for urine PCA3 is commercially available (PROGENSA PCA3 assay, Gen-Probe Inc., San Diego, CA), and the company has submitted a PMA application to the FDA.

Currently, 2 potential clinical uses of this marker are undergoing extensive evaluation. The first (the indication for which the PROGENSA assay is currently being considered by the FDA) is to help determine which of the men with elevated serum PSA(KLK3) and who have already undergone one negative biopsy should undergo a repeat biopsy. The second potential indication is to guide initial biopsy decisions.

In terms of guiding decisions for a repeat biopsy, a large number of studies have been performed, most of which have suggested that the PCA3 test is more specific for predicting a positive repeat biopsy than are serum PSA(KLK3) levels or changes in PSA(KLK3) over time.[179] Furthermore, a recent study was conducted from the placebo arm of the REDUCE trial, which was a prospective randomized clinical trial to determine whether dutasteride was associated with a decreased risk of prostate cancer after 2 or 4 years.[180] In this study, the PCA3 scores were measurable from 1072 of the 1140 subjects, for a 94% informative rate. The use of PCA3 in combination with serum PSA(KLK3) and other risk factors was found to significantly increase diagnostic accuracy in predicting a positive biopsy. These results, using this analytically validated assay, indicate a level of evidence of IB. The clinical utility of PCA3 testing must be interpreted with caution, because the impact of refining decisions regarding when to perform repeat diagnostic biopsies has not been shown.

Several studies have also shown the PCA3 has a high specificity for predicting an initial positive biopsy in men with elevated PSA(KLK3) levels (studies usually conducted in men with serum PSA[KLK3] levels of 4–10 ng/mL).[179] Furthermore, this marker was recently evaluated in a European, prospective, multicenter study in which men with a serum PSA(KLK3) level of 2.5 to 10 ng/mL all underwent prostate needle biopsies.[181] The study was considered a "real life clinical practice study" by the authors and consisted of 516 men. ROC curves showed a significantly higher area under the curve for the PCA3 score versus total PSA(KLK3), PSA(KLK3) density, and percent free PSA(KLK3). Furthermore, the PCA3 score was significantly higher in men with a biopsy Gleason score of 7 or greater versus those with a score less than 7, and in men in whom greater than 33% of the needle cores were positive for cancer versus men with fewer than 33% of cores positive. Overall the inclusion of the PCA3 in multivariable models increased the predictive accuracy by up to 5.5%. These results, therefore, also indicate level IB clinical validity, albeit with a modest improvement in diagnostic accuracy and insufficient evidence to determine the true clinical utility of this assay in routine clinical management.

CTCs

The CellSearch CTC detection system has been cleared for use in metastatic prostate cancer by the FDA. However, this test is not included in the NCCN Guidelines for Prostate Cancer (available at www.NCCN.org). The test uses an immunomagnetic system to isolate cells with the features of epithelial cells from the peripheral blood and provides an absolute count of CTC per 7.5 mL blood sample. Presence of greater than 5 CTCs per 7.5 mL sample at baseline measurement has been shown to

be associated with shortened overall survival,[182] an effect which is seen through the course of several CTC measurements. The association with elevated levels of CTCs and poor prognosis is consistent and strong, and therefore the level of evidence is IIB. In addition, change in numbers of CTCs in response to therapy has recently met the Prentice criterion of surrogacy for overall survival in the setting of treatment with abiraterone.[182] Thus, decreased CTC levels from greater than 5 to lower than 5 is a predictive biomarker, although it is only informative once therapy has begun, with a level of evidence of IIA. Currently, the clinical utility of changing CTC levels remains untested, because no trial has evaluated whether changing therapy based on a lack of CTC response improves outcome.

Similar to other forms of cancer, comprehensive molecular analyses of prostate cancer using microarray and sequencing technologies have resulted in a greater understanding of tumor biology. It remains clear that the androgen receptor is a driver of both treatment-naive and castration-resistant prostate cancer, but biomarkers that accurately reflect androgen receptor activity have yet to be developed. Similarly, most prostate cancers contain fusion proteins between androgen-regulated genes and members of the ETS family of transcription factors.[183] These fusion genes are likely also oncogenic drivers, but data conflict regarding their prognostic strength as biomarkers.[184]

Molecular Testing in Acute Myeloid Leukemia

Acute myeloid leukemia (AML) is a genetically and clinically heterogeneous neoplasm characterized by the accumulation of genetic and epigenetic alterations in hematopoietic progenitor cells that alter normal mechanisms of differentiation, proliferation, and survival.[185] Cytogenetics have been used for outcome prediction and treatment guidance, because they were shown to enable patients with AML to be stratified into cytogenetic risk groups (i.e., favorable, intermediate, and unfavorable) based on the presence of nonrandom chromosome aberrations or normal karyotype.[186] However, within each cytogenetic group, it has become clear that molecular heterogeneity exists, which can be further used to determine molecular risk stratification and treatment selection.[187,188]

Few of the genetic molecular alterations have shown relevance to the biologic and clinical classification of AML as first recognized by the WHO classification,[189] and more recently have been incorporated into clinical guidelines such as the NCCN Guidelines for AML[190] and the European LeukemiaNet[191] guidelines. Consensus exists regarding clinical impact for at least for 4 of the markers (*NPM1*, *CEBPA*, and *KIT* mutations, and *FLT3* internal tandem duplication [ITD]). However, the best therapeutic approaches for patients harboring these mutations remain to be defined. Table 10 summarizes current molecular biomarkers in AML.

NPM1

Mutations in exon 12 of the *NPM1* gene are found in approximately one-third of adult patients with AML, and result in abnormal cytoplasmic localization of the NPM1 protein.[192] *NPM1* mutations are frequent in cytogenetically normal AML (CN-AML) but can also be found in other cytogenetic or molecular groups, most frequently in patients harboring *FLT3* mutations.[193] *NPM1* mutations, particularly the genotype "mutated *NPM1* without concurrent *FLT3*-ITD," has been associated with achievement of complete remission and favorable outcome. Recent data also suggest that *NPM1* mutations seem to predict better outcome in older patients (> 60 years of age), and perhaps could be used to identify individuals in this age group who may benefit from intensive conventional chemotherapy.[194] However, these results require validation.

CEBPA

Approximately 15% of patients with CN-AML harbor *CEBPA* mutations.[195] These mutations, which may also be found in other cytogenetic groups of AML, usually affect the *N*-terminal region of the gene, thereby resulting in a mutant protein similar to a truncated CEBPA isoform found also in normal cells or the C-terminal basic region and leucine zipper domain of the gene. Both mutations may result in a mutant protein with altered transcription factor activity with respect to the wild-type counterpart. In approximately two-thirds of cases, *C*- and *N*-terminal mutations are "biallelic" mutations (also called *double mutations*), with most (~90%) being compound heterozygous (C-terminal on one allele and *N*-terminal on the other) and the rest being homo-

Table 10 Current Molecular Biomarkers in Acute Myeloid Leukemia

Biomarker	Molecular Compartment	Purpose	Analytic Validity Demonstrated	Level of Evidence	NCCN Category of Evidence	References
Markers With Accepted Clinical Utility						
FLT3-ITD	Tumor DNA	Predictive/prognostic: *FLT3*-ITD mutation confers poor risk status	Multiple methods: PCR, multiplex assays, direct sequencing	IIB	Prognostic: 2A Predictive: 2B	Schlenk et al.[193]
CEBPA mutation	Tumor DNA	Predictive/prognostic: *CEBPA* mutation with normal cytogenetics and in absence of *FLT3*-ITD confers better risk status	Multiple methods: PCR, multiplex assays, direct sequencing	IIB	Prognostic: 2A Predictive: 2B	Marcucci et al.[197] Burnett et al.[196] Taskesen et al.[198] Wouters et al.[199]
NPM1 mutation	Tumor DNA	Predictive/prognostic: *NPM1* mutation with normal cytogenetics and in absence of *FLT3*-ITD confers better risk status	Multiple methods: PCR, multiplex assays, direct sequencing	IIB	Prognostic: 2A Predictive: 2B	Becker et al.[194]
KIT mutation	Tumor DNA	Predictive/prognostic c-KIT mutations in the presence of t(8;21), inv(16), or t(16;16) confers a higher risk of relapse	Multiple methods: PCR, multiplex assays, direct sequencing	IIB	Prognostic: 2A Predictive: 2B	Paschka[200] Paschka et al.[201]
Markers With Emerging Evidence						
FLT3-TKD mutation	Tumor DNA	Prognostic relevance of *FLT3*-TKD mutations is controversial	Multiple methods: PCR, multiplex assays, direct sequencing	Insufficient data		Schlenk et al.[193]
WT1 mutation	Tumor DNA	*WT1* mutations associated with poorer outcome	Multiple methods: PCR, multiplex assays, direct sequencing	IIB		Paschka et al.[205] Virappane et al.[206] Gaidzik et al.[204]
RUNX1 mutation	Tumor DNA	*RUNX1* mutations associated with poorer outcome	Multiple methods: PCR, multiplex assays, direct sequencing	IIB		Tang et al.[203]
MLL-PTD	Tumor DNA	*MLL*-PTD associated with inferior CR duration and relapse free survival	RT-PCR	IIB		Whitman et al.[202]
IDH1 mutation	Tumor DNA	*IDH1* mutations associated with *NPM1* mutation and predict worse outcome for patients with mutated *NPM1* without *FLT3*-ITD	Multiple methods: PCR, multiplex assays, direct sequencing	IIB		Mardis et al.[187] Marcucci et al.[207] Paschka et al.[208]
IDH2 c.515G>A p. R172K	Tumor DNA	*IDH2 R172* may confer lower probability of achieving CR and possibly also inferior outcome	Multiple methods: PCR, multiplex assays, direct sequencing	IIB		Mardis et al.[187] Marcucci et al.[207] Paschka et al.[208]
IDH2 codon 140 mutation	Tumor DNA	prognostic relevance of *IDH2* codon 140 mutation is controversial	Multiple methods: PCR, multiplex assays, direct sequencing	IIB		Mardis et al.[187] Marcucci et al.[207] Paschka et al.[208]

Abbreviations: CR, complete remission; ITD, internal tandem duplication; PCR, polymerase chain reaction; PTD, partial tandem duplication; RT-PCR, reverse transcriptase polymerase chain reaction; TKD, tyrosine kinase domain.

zygous (either both C- or *N*-terminal).[196–198] *CEBPA* mutations have consistently been associated with a relatively favorable outcome for patients with CN-AML.[196–198] However, more recent studies indicate that the favorable impact is restricted to patients with double mutations.[199]

Patients with *CEBPA* mutations are grouped together with those with AML with mutated *NPM1* without *FLT3*-ITD and those with core binding factor (CBF) AML [t(8;21) or inv(16)/t(16;16)] in a favorable-risk group.[191] Therapeutic recommendations for these subsets are similar and consist of chemotherapy-based approaches, whereas allogeneic hematopoietic stem cell transplantation (HSCT) should be reserved for only patients who have experienced relapse or have proven additionally adverse cytogenetic or molecular features.

FLT3

Two types of mutations that result in the constitutive activation of the encoded tyrosine kinase receptor protein have been identified in the *FLT3* gene: tandem internal duplications within the juxtamembrane domain and point mutations with the tyrosine kinase domain (TKD). The *FLT3*-ITD is found in approximately 20% of unselected patients with AML and approximately 30% of those with CN-AML.[193] The prognosis of patients with CN-AML with *FLT3*-ITD is significantly inferior compared with CN-AML without the mutation when treated with current standard chemotherapy, particularly if the ratio of mutated versus wild-type alleles is high.[193] The prognostic relevance of *FLT3*-TKD mutations instead is controversial. Although patients with *FLT3*-ITD have been reported to benefit from allogeneic HSCT, this approach must be validated prospectively. Furthermore, because *FLT3*-ITD encodes a constitutively activated tyrosine kinase protein, small inhibitor molecules combined with chemotherapy are being tested in phase III clinical trials.

KIT

KIT mutations are found in 25% to 30% of CBF-AML and are rare in other AML subsets.[200] In most studies, *KIT* mutations have been associated with inferior outcome.[201] Notably, *KIT* is not only mutated but also expressed at significantly higher levels in CBF-AML compared with other AML subsets. Currently no data support the use of *KIT* mutational status to guide therapy, although these patients may benefit from more-aggressive treatment with allogeneic HSCT. Clinical trials are currently underway evaluating KIT inhibitors in CBF-AML.

For use as prognostic markers, *FLT3*-ITD, *KIT*, *CEBPA*, and *NPM1* mutations are all given an NCCN category of 2A. As predictive markers, all have an NCCN category of 2B. Multiple large-scale retrospective studies of marker expression and outcome for these mutations gives them a combined level of evidence of IIB.

Other Gene Mutations

In addition to the aforementioned mutations, other genetic mutations have been found in patients with AML. These mutations either do not seem to significantly contribute to risk stratification or have so far been less well studied for their prognostic significance.

MLL: Partial tandem duplications (PTD) of *MLL* are found in 5% to 11% of patients with CN-AML and frequently in AML with trisomy 11. *MLL*-PTD have been associated with inferior complete remission duration and relapse-free survival, although more recent studies show no prognostic impact in patients with CN-AML intensively treated with autologous HSCT or 4 cycles of chemotherapy consolidations.[202]

RUNX1: *RUNX1* is deregulated in AML by chromosomal translocations and by mutations clustering in the Runt domain of the gene. *RUNX1* mutations have been associated with undifferentiated (M0) morphology, and with specific chromosome aberrations, such as trisomy 21 and trisomy 13. In recent studies, *RUNX1* mutations were found in approximately 13% of patients with AML and associated with worse outcome.[203]

WT1: *WT1* mutations are found in 10% to 13% of patients with CN-AML. In most studies, *WT1* mutations have been associated with inferior outcome.[204–206]

IDH1/IDH2: Mutations of *IDH1* and *IDH2* were first reported in gliomas, and only more recently in AML.[187] The aggregate frequency of *IDH1* and *IDH2* mutations in AML is relatively high, with approximately 15% to 20% of all patients with AML and 25% to 30% of patients with CN-AML harboring either *IDH1* or *IDH2* mutations.[207,208] Initial studies from larger and homogeneous cohorts of patients indicate that *IDH1* mutations are significantly associated with *NPM1* mutations and predict worse outcome for patients with mutated *NPM1* without

FLT3-ITD. The distinct *R172 IDH2* mutation is rarely associated with any of the other known prognostic mutations, and seems to confer lower probability of achieving complete remission and possibly also inferior outcome. The prognostic impact of *IDH2* mutation in codon 140 remains instead controversial.

Testing for mutations in these genes is not included in the NCCN Guidelines for AML (available at www.NCCN.org). However, results of large-scale retrospective analyses in samples from clinical trials gives each of them a combined level of evidence score of IIB. Clinical utility remains to be determined.

***NRAS, TP53, TET2, DNMT3A,* and *ASXL1*:** *NRAS*, *TP53*, *TET2*, *DNMT3A*, and *ASXL1* mutations have also been found in patients with AML, but their clinical impact as prognosticators or predictors of response to distinct treatment approaches remains to be fully defined.[209–214] These mutations, which have correspondingly fewer data indicating their prognostic or predictive role, are all assigned a combined level of evidence of IIIC for clinical validity.

A continuing challenge in validating biomarkers in AML, as each new marker further divides patients into smaller phenotypic groups, will be to find sufficient numbers of patients to establish the clinical utility of each marker and to discover the impact that each may have on treatment or prognosis.

Conclusions

Cancer care is becoming increasingly dependent on tumor markers to diagnose, anticipate prognosis, and select optimal therapy for patients. Although knowledge of the specific tumor markers that have the greatest value in care will evolve over time, it is important to develop a common language with respect to the specific purpose of each tumor marker, the steps in developing a tumor marker, and the different levels of evidence supporting the specific use of a tumor marker. The task force was established to review each of these processes and to identify the tumor markers that are most pertinent today.

This report presents tumor markers for 6 cancer types and highlights some of the challenges faced by oncologists in determining which tests will provide the most useful information to direct patient care. First, although significant efforts have been made to standardize the development and reporting of diagnostic, prognostic, and predictive tumor markers, best practices have not been broadly adopted and the literature supporting the use of tumor markers is often incomplete or reported in a manner that hampers comparisons across studies. Second, the actual developmental process for tumor markers and the regulatory oversight of that process is relatively immature compared with drug development and, as a result, confusion often occurs regarding the steps required to incorporate tumor markers into care. Third, several technical methods are often available to measure a specific molecular marker, usually with concordance between assays but each with distinct analytic performance characteristics and few opportunities for rigorous head-to-head comparison. Fourth, great interest has been shown among all stakeholders in cancer care, including clinical and basic investigators, providers, and patients, to rapidly incorporate emerging biologic insights into clinical care that drives a desire for early adoption, often before rigorous assessment of the analytic validity, clinical validity, and clinical utility of a tumor marker.

With these challenges in mind, this report focuses on presenting the different uses for tumor markers, an overview of the different steps of tumor marker development, and an adapted, simplified categorization of level of evidence. After reviewing the work previously reported on tumor markers, the task force believes that identifying the pertinent references and efforts previously focused on biomarker standardization and classification of levels of evidence for biomarkers is an important first step. Admittedly, challenges remain with respect to the development of a simple yet comprehensive classification system for levels of evidence. The current systems focus only on the supportive data and not on panel consensus, as in the NCCN Guidelines. With time, the classification of level of evidence for tumor markers and the common accepted criteria will likely be increasingly refined.

Looking forward, the task force believes that the community interested in developing tumor markers should consider and adopt the following recommendations:

- Assure a test is analytically valid before performing rigorous testing for clinical validity.
- If the test is a qualitative one, such as immunohistochemistry, determine the degree to which the test has been rigorously quality controlled.

Have preferred methods or protocols been published?

- When evaluating new tests, check to see if BRISQ standards or similar have been used for preanalytic sample collection in those trials.
- When evaluating new studies, check to see if REMARK standards were used in clinical trial design for marker study and reporting of the results.
- Consider whether the test as it currently exists can be scaled up for general use, is an established LDT in a CLIA-certified laboratory, or is an FDA-approved/cleared test.
- Include comparisons of testing methods and make recommendations for preferred methods.
- Include and consider recommendations from other groups, including CAP, ASCO, and EGAPP. If recommendations/outcomes differ, address them specifically so that clinicians can understand the source of potential disagreement, and thus improve decision-making.
- Incorporate a formal discussion of levels of evidence[32,91] (Tables 1–3) to determine clinical utility for tests under consideration.
- Include a discussion of needed studies or evidence for tests under consideration if gaps are perceived in the literature.

The use of tumor markers will continue to transform cancer care. Only through adopting a common language and standards of validation will timely and definitive studies on emerging markers be effectively communicated, thereby ensuring that clinicians take full advantage of the current genomic era.

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References:

1. American Society of Clinical Oncology policy statement update: genetic testing for cancer susceptibility. J Clin Oncol 2003;21:2397–2406.
2. Haber DA, Gray NS, Baselga J. The evolving war on cancer. Cell 2011;145:19–24.
3. Khleif SN, Doroshow JH, Hait WN; AACR-FDA-NCI Cancer Biomarkers Collaborative. AACR-FDA-NCI Cancer Biomarkers Collaborative consensus report: advancing the use of biomarkers in cancer drug development. Clin Cancer Res 2010;16:3299–3318.
4. Marchio C, Dowsett M, Reis-Filho JS. Revisiting the technical validation of tumour biomarker assays: how to open a Pandora's box. BMC Med 2011;9:41.
5. McShane LM, Altman DG, Sauerbrei W, et al. Reporting recommendations for tumor marker prognostic studies. J Clin Oncol 2005;23:9067–9072.
6. Saijo N. Critical comments for roles of biomarkers in the diagnosis and treatment of cancer. Cancer Treat Rev, in press.
7. Simon R. Clinical trial designs for evaluating the medical utility of prognostic and predictive biomarkers in oncology. Per Med 2010;7:33–47.
8. Faderl S, Talpaz M, Estrov Z, et al. The biology of chronic myeloid leukemia. N Engl J Med 1999;341:164–172.
9. Goldstein I, Marcel V, Olivier M, et al. Understanding wild-type and mutant p53 activities in human cancer: new landmarks on the way to targeted therapies. Cancer Gene Ther 2011;18:2–11.
10. Joensuu H, Kellokumpu-Lehtinen PL, Bono P, et al. Adjuvant docetaxel or vinorelbine with or without trastuzumab for breast cancer. N Engl J Med 2006;354:809–820.
11. Piccart-Gebhart MJ, Procter M, Leyland-Jones B, et al. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. N Engl J Med 2005;353:1659–1672.
12. Romond EH, Perez EA, Bryant J, et al. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. N Engl J Med 2005;353:1673–1684.
13. Chapman PB, Hauschild A, Robert C, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med 2011;364:2507–2516.
14. Pollock PM, Meltzer PS. A genome-based strategy uncovers frequent BRAF mutations in melanoma. Cancer Cell 2002;2:5–7.
15. Zelboraf. Available at: http://www.gene.com/gene/products/information/zelboraf/pdf/pi.pdf. Accessed September 30, 2011.
16. Teutsch SM, Bradley LA, Palomaki GE, et al. The Evaluation of Genomic Applications in Practice and Prevention (EGAPP) initiative: methods of the EGAPP Working Group. Genet Med 2009;11:3–14.
17. Bordeaux J, Welsh A, Agarwal S, et al. Antibody validation. Biotechniques 2010;48:197–209.
18. Wu YL, Dudognon C, Nguyen E, et al. Immunodetection of human telomerase reverse-transcriptase (hTERT) re-appraised: nucleolin and telomerase cross paths. J Cell Sci 2006;119:2797–2806.
19. Moore HM, Kelly AB, Jewell SD, et al. Biospecimen reporting for improved study quality (BRISQ). Cancer Cytopathol 2011;119:92–101.
20. McShane LM, Altman DG, Sauerbrei W, et al. Reporting recommendations for tumor marker prognostic studies (REMARK). J Natl Cancer Inst 2005;97:1180–1184.
21. Shedden K, Taylor JM, Enkemann SA, et al. Gene expression-based survival prediction in lung adenocarcinoma: a multi-site, blinded validation study. Nat Med 2008;14:822–827.
22. Xie Y, Minna JD. Predicting the future for people with lung cancer. Nat Med 2008;14:812–813.
23. Edge SB, Byrd DR, Compton CC, et al. AJCC Cancer Staging

Manual, 7th ed. New York: Springer-Verlag; 2009.

24. Engstrom PF, Bloom MG, Demetri GD, et al. NCCN Molecular Testing White Paper: effectiveness, efficiency, and reimbursement. J Natl Compr Canc Netw, in press.
25. The Medical Device Amendments of 1976. Publ L No. 94-295, 90 Stat 539 (May 28, 1976).
26. FDA Rule on Analyte Specific Reagents, 62 Fed Regist 62,243, 62,249 (November 21, 1997). 1997.
27. US Food and Drug Administration. Draft Guidance for Industry and Food and Drug Administration Staff. In Vitro Companion Diagnostic Devices. Available at: http://www.fda.gov/medicaldevices/deviceregulationandguidance/guidancedocuments/ucm262292.htm. Accessed October 10, 2011.
28. US Food and Drug Administration. Draft Guidance for Industry, Clinical Laboratories, and FDA Staff. In Vitro Diagnostic Multivariate Index Assays. Available at: www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm071455.htm. Accessed October 10, 2011.
29. Freidlin B, McShane LM, Korn EL. Randomized clinical trials with biomarkers: design issues. J Natl Cancer Inst 2010;102:152–160.
30. Sargent DJ, Conley BA, Allegra C, Collette L. Clinical trial designs for predictive marker validation in cancer treatment trials. J Clin Oncol 2005;23:2020–2027.
31. Hayes DF, Bast RC, Desch CE, et al. Tumor marker utility grading system: a framework to evaluate clinical utility of tumor markers. J Natl Cancer Inst 1996;88:1456–1466.
32. Simon RM, Paik S, Hayes DF. Use of archived specimens in evaluation of prognostic and predictive biomarkers. J Natl Cancer Inst 2009;101:1446–1452.
33. Amado RG, Wolf M, Peeters M, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol 2008;26:1626–1634.
34. Karapetis CS, Khambata-Ford S, Jonker DJ, et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. N Engl J Med 2008;359:1757–1765.
35. Brem SS, Bierman PJ, Brem H, et al. NCCN Clinical Practice Guidelines in Oncology: Central Nervous System Cancers. Version 2, 2011. Available at: http://www.nccn.org/professionals/physician_gls/pdf/cns.pdf. Accessed October 10, 2011.
36. Bourne TD, Schiff D. Update on molecular findings, management and outcome in low-grade gliomas. Nat Rev Neurol 2010;6:695–701.
37. Hoang-Xuan K, Capelle L, Kujas M, et al. Temozolomide as initial treatment for adults with low-grade oligodendrogliomas or oligoastrocytomas and correlation with chromosome 1p deletions. J Clin Oncol 2004;22:3133–3138.
38. Buckner JC, Gesme D Jr, O'Fallon JR, et al. Phase II trial of procarbazine, lomustine, and vincristine as initial therapy for patients with low-grade oligodendroglioma or oligoastrocytoma: efficacy and associations with chromosomal abnormalities. J Clin Oncol 2003;21:251–255.
39. Cairncross G, Macdonald D, Ludwin S, et al. Chemotherapy for anaplastic oligodendroglioma. National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol 1994;12:2013–2021.
40. Ino Y, Betensky RA, Zlatescu MC, et al. Molecular subtypes of anaplastic oligodendroglioma: implications for patient management at diagnosis. Clin Cancer Res 2001;7:839–845.
41. Kaloshi G, Benouaich-Amiel A, Diakite F, et al. Temozolomide for low-grade gliomas: predictive impact of 1p/19q loss on response and outcome. Neurology 2007;68:1831–1836.
42. van den Bent M, Chinot OL, Cairncross JG. Recent developments in the molecular characterization and treatment of oligodendroglial tumors. Neuro Oncol 2003;5:128–138.
43. Franco-Hernandez C, Martinez-Glez V, de Campos JM, et al. Allelic status of 1p and 19q in oligodendrogliomas and glioblastomas: multiplex ligation-dependent probe amplification versus loss of heterozygosity. Cancer Genet Cytogenet 2009;190:93–96.
44. Idbaih A, Kouwenhoven M, Jeuken J, et al. Chromosome 1p loss evaluation in anaplastic oligodendrogliomas. Neuropathology 2008;28:440–443.
45. Jeuken J, Cornelissen S, Boots-Sprenger S, et al. Multiplex ligation-dependent probe amplification: a diagnostic tool for simultaneous identification of different genetic markers in glial tumors. J Mol Diagn 2006;8:433–443.
46. Smith JS, Alderete B, Minn Y, et al. Localization of common deletion regions on 1p and 19q in human gliomas and their association with histological subtype. Oncogene 1999;18:4144–4152.
47. Woehrer A, Sander P, Haberler C, et al. FISH-based detection of 1p 19q codeletion in oligodendroglial tumors: procedures and protocols for neuropathological practice—a publication under the auspices of the Research Committee of the European Confederation of Neuropathological Societies (Euro-CNS). Clin Neuropathol 2011;30:47–55.
48. Parsons DW, Jones S, Zhang X, et al. An integrated genomic analysis of human glioblastoma multiforme. Science 2008;321:1807–1812.
49. Houillier C, Wang X, Kaloshi G, et al. IDH1 or IDH2 mutations predict longer survival and response to temozolomide in low-grade gliomas. Neurology 2010;75:1560–1566.
50. von Deimling A, Korshunov A, Hartmann C. The next generation of glioma biomarkers: MGMT methylation, BRAF fusions and IDH1 mutations. Brain Pathol 2011;21:74–87.
51. Dubbink HJ, Taal W, van Marion R, et al. IDH1 mutations in low-grade astrocytomas predict survival but not response to temozolomide. Neurology 2009;73:1792–1795.
52. Capper D, Weissert S, Balss J, et al. Characterization of R132H mutation-specific IDH1 antibody binding in brain tumors. Brain Pathol 2010;20:245–254.
53. Capper D, Zentgraf H, Balss J, et al. Monoclonal antibody specific for IDH1 R132H mutation. Acta Neuropathol 2009;118:599–601.
54. van den Bent MJ, Dubbink HJ, Marie Y, et al. IDH1 and IDH2 mutations are prognostic but not predictive for outcome in anaplastic oligodendroglial tumors: a report of the European Organization for Research and Treatment of Cancer Brain Tumor Group. Clin Cancer Res 2010;16:1597–1604.
55. Hartmann C, Hentschel B, Wick W, et al. Patients with IDH1 wild type anaplastic astrocytomas exhibit worse prognosis than IDH1-mutated glioblastomas, and IDH1 mutation status accounts for the unfavorable prognostic effect of higher age: implications for classification of gliomas. Acta Neuropathol 2010;120:707–718.
56. English DR, Young JP, Simpson JA, et al. Ethnicity and risk for colorectal cancers showing somatic BRAF V600E mutation or CpG island methylator phenotype. Cancer Epidemiol Biomarkers Prev 2008;17:1774–1780.
57. Limsui D, Vierkant RA, Tillmans LS, et al. Cigarette smoking and colorectal cancer risk by molecularly defined subtypes. J Natl Cancer Inst 2010;102:1012–1022.

58. Ogino S, Nosho K, Kirkner GJ, et al. CpG island methylator phenotype, microsatellite instability, BRAF mutation and clinical outcome in colon cancer. Gut 2009;58:90–96.

59. Jones DT, Kocialkowski S, Liu L, et al. Tandem duplication producing a novel oncogenic BRAF fusion gene defines the majority of pilocytic astrocytomas. Cancer Res 2008;68:8673–8677.

60. Aldape KD, Wang M, Sulman EP, et al. RTOG 0525: molecular correlates from a randomized phase III trial of newly diagnosed glioblastoma [abstract]. J Clin Oncol 2011;29(Suppl 1):Abstract LBA2000.

61. Hegi ME, Diserens AC, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 2005;352:997–1003.

62. Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol 2009;10:459–466.

63. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005;352:987–996.

64. Weller M, Stupp R, Reifenberger G, et al. MGMT promoter methylation in malignant gliomas: ready for personalized medicine? Nat Rev Neurol 2010;6:39–51.

65. Noushmehr H, Weisenberger DJ, Diefes K, et al. Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. Cancer Cell 2010;17:510–522.

66. Toyota M, Yamamoto E. DNA methylation changes in cancer. Prog Mol Biol Transl Sci 2011;101:447–457.

67. Hilsenbeck SG, Ravdin PM, de Moor CA, et al. Time-dependence of hazard ratios for prognostic factors in primary breast cancer. Breast Cancer Res Treat 1998;52:227–237.

68. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet 2005;365:1687–1717.

69. Dowsett M, Allred C, Knox J, et al. Relationship between quantitative estrogen and progesterone receptor expression and human epidermal growth factor receptor 2 (HER2) status with recurrence in the Arimidex, Tamoxifen, Alone or in Combination trial. J Clin Oncol 2008;26:1059–1065.

70. Viale G, Regan MM, Maiorano E, et al. Prognostic and predictive value of centrally reviewed expression of estrogen and progesterone receptors in a randomized trial comparing letrozole and tamoxifen adjuvant therapy for postmenopausal early breast cancer: BIG 1-98. J Clin Oncol 2007;25:3846–3852.

71. Viale G, Regan MM, Maiorano E, et al. Chemoendocrine compared with endocrine adjuvant therapies for node-negative breast cancer: predictive value of centrally reviewed expression of estrogen and progesterone receptors—International Breast Cancer Study Group. J Clin Oncol 2008;26:1404–1410.

72. Carlson RW, Allred DC, Anderson BO, et al. NCCN Clinical Practice Guidelines in Oncology: Breast Cancer. Version 2, 2011. Available at: http://www.nccn.org/professionals/physician_gls/pdf/breast.pdf. Accessed October 18, 2011.

73. Allred DC, Carlson RW, Berry DA, et al. NCCN Task Force Report: estrogen receptor and progesterone receptor testing in breast cancer by immunohistochemistry. J Natl Compr Canc Netw 2009;7(Suppl 6):S1–21; quiz S22–23.

74. Hammond ME, Hayes DF, Dowsett M, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. Arch Pathol Lab Med 2010;134:907–922.

75. Hammond ME, Hayes DF, Dowsett M, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. J Clin Oncol 2010;28:2784–2795.

76. Hammond ME, Hayes DF, Wolff AC. Clinical notice for American Society of Clinical Oncology-College of American Pathologists guideline recommendations on ER/PgR and HER2 testing in breast cancer. J Clin Oncol 2011;29:e458.

77. Carlson RW, Moench SJ, Hammond ME, et al. HER2 testing in breast cancer: NCCN Task Force report and recommendations. J Natl Compr Canc Netw 2006;4(Suppl 3):S1–22; quiz S23–24.

78. Wolff AC, Hammond ME, Schwartz JN, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. J Clin Oncol 2007;25:118–145.

79. Albain KS, Barlow WE, Shak S, et al. Prognostic and predictive value of the 21-gene recurrence score assay in postmenopausal women with node-positive, oestrogen-receptor-positive breast cancer on chemotherapy: a retrospective analysis of a randomised trial. Lancet Oncol 2010;11:55–65.

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

81. Paik S, Tang G, Shak S, et al. Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer. J Clin Oncol 2006;24:3726–3734.

82. Harris L, Fritsche H, Mennel R, et al. American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. J Clin Oncol 2007;25:5287–5312.

83. Recommendations from the EGAPP Working Group: genetic testing strategies in newly diagnosed individuals with colorectal cancer aimed at reducing morbidity and mortality from Lynch syndrome in relatives. Genet Med 2009;11:35–41.

84. Recommendations from the EGAPP Working Group: can tumor gene expression profiling improve outcomes in patients with breast cancer? Genet Med 2009;11:66–73.

85. Hormone therapy with or without combination chemotherapy in treating women who have undergone surgery for node-negative breast cancer (the TAILORx trial). Available at: http://clinicaltrials.gov/ct2/show/NCT00310180. Accessed September 30, 2011.

86. Tamoxifen citrate, letrozole, anastrozole, or exemestane with or without chemotherapy in treating patients with invasive RxPONDER breast cancer. Available at: http://clinicaltrials.gov/ct2/show/NCT01272037. Accessed September 30, 2011.

87. van 't Veer LJ, Dai H, van de Vijver MJ, et al. Gene expression profiling predicts clinical outcome of breast cancer. Nature 2002;415:530–536.

88. van de Vijver MJ, He YD, van 't Veer LJ, et al. A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med 2002;347:1999–2009.

89. Cardoso F, Van't Veer L, Rutgers E, et al. Clinical application of the 70-gene profile: the MINDACT trial. J Clin Oncol 2008;26:729–735.

90. Cristofanilli M, Budd GT, Ellis MJ, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med 2004;351:781–791.

91. Hayes DF, Cristofanilli M, Budd GT, et al. Circulating tumor cells at each follow-up time point during therapy of metastatic breast cancer patients predict progression-free and overall survival. Clin Cancer Res 2006;12:4218–4224.

92. Brink M, Weijenberg MP, De Goeij AF, et al. Fat and K-ras mutations in sporadic colorectal cancer in The Netherlands Cohort Study. Carcinogenesis 2004;25:1619–1628.

93. Nosho K, Irahara N, Shima K, et al. Comprehensive biostatistical analysis of CpG island methylator phenotype in colorectal cancer using a large population-based sample. PLoS One 2008;3:e3698.

94. Samowitz WS, Curtin K, Schaffer D, et al. Relationship of Ki-ras mutations in colon cancers to tumor location, stage, and survival: a population-based study. Cancer Epidemiol Biomarkers Prev 2000;9:1193–1197.

95. Bokemeyer C, Kohne C, Rougier P, et al. Cetuximab with chemotherapy (CT) as first-line treatment for metastatic colorectal cancer (mCRC): analysis of the CRYSTAL and OPUS studies according to KRAS and BRAF mutation status [abstract]. J Clin Oncol 2010;28(Suppl 1):Abstract 3506.

96. Van Cutsem E, Kohne CH, Hitre E, et al. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N Engl J Med 2009;360:1408–1417.

97. Benson AB III, Amoletti JP, Bekaii-Saab T, et al. NCCN Clinical Practice Guidelines in Oncology: Colon Cancer. Version 1, 2012. Available at: http://www.nccn.org/professionals/physician_gls/pdf/colon.pdf. Accessed October 18, 2011.

98. Allegra CJ, Jessup JM, Somerfield MR, et al. American Society of Clinical Oncology provisional clinical opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. J Clin Oncol 2009;27:2091–2096.

99. De Roock W, Jonker DJ, Di Nicolantonio F, et al. Association of KRAS p.G13D mutation with outcome in patients with chemotherapy-refractory metastatic colorectal cancer treated with cetuximab. JAMA 2010;304:1812–1820.

100. Tejpar S, Bokemeyer C, Celik I, et al. Influence of KRAS G13D mutations on outcome in patients with metastatic colorectal cancer (mCRC) treated with first-line chemotherapy with or without cetuximab [abstract]. J Clin Oncol 2011;29(Suppl 1):Abstract 3511.

101. de Vogel S, Weijenberg MP, Herman JG, et al. MGMT and MLH1 promoter methylation versus APC, KRAS and BRAF gene mutations in colorectal cancer: indications for distinct pathways and sequence of events. Ann Oncol 2009;20:1216–1222.

102. Samowitz WS, Albertsen H, Herrick J, et al. Evaluation of a large, population-based sample supports a CpG island methylator phenotype in colon cancer. Gastroenterology 2005;129:837–845.

103. De Roock W, Claes B, Bernasconi D, et al. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. Lancet Oncol 2010;11:753–762.

104. Di Nicolantonio F, Martini M, Molinari F, et al. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. J Clin Oncol 2008;26:5705–5712.

105. Tol J, Nagtegaal ID, Punt CJ. BRAF mutation in metastatic colorectal cancer. N Engl J Med 2009;361:98–99.

106. Roth AD, Tejpar S, Delorenzi M, et al. Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: results of the translational study on the PETACC-3, EORTC 40993, SAKK 60-00 trial. J Clin Oncol 2010;28:466–474.

107. Samowitz WS, Sweeney C, Herrick J, et al. Poor survival associated with the BRAF V600E mutation in microsatellite-stable colon cancers. Cancer Res 2005;65:6063–6069.

108. Ogino S, Kawasaki T, Brahmandam M, et al. Sensitive sequencing method for KRAS mutation detection by pyrosequencing. J Mol Diagn 2005;7:413–421.

109. Tsiatis AC, Norris-Kirby A, Rich RG, et al. Comparison of Sanger sequencing, pyrosequencing, and melting curve analysis for the detection of KRAS mutations: diagnostic and clinical implications. J Mol Diagn 2010;12:425–432.

110. Arcila M, Lau C, Nafa K, Ladanyi M. Detection of KRAS and BRAF mutations in colorectal carcinoma roles for high-sensitivity locked nucleic acid-PCR sequencing and broad-spectrum mass spectrometry genotyping. J Mol Diagn 2011;13:64–73.

111. Ogino S, Cantor M, Kawasaki T, et al. CpG island methylator phenotype (CIMP) of colorectal cancer is best characterised by quantitative DNA methylation analysis and prospective cohort studies. Gut 2006;55:1000–1006.

112. Weisenberger DJ, Siegmund KD, Campan M, et al. CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. Nat Genet 2006;38:787–793.

113. Des Guetz G, Schischmanoff O, Nicolas P, et al. Does microsatellite instability predict the efficacy of adjuvant chemotherapy in colorectal cancer? A systematic review with meta-analysis. Eur J Cancer 2009;45:1890–1896.

114. Guastadisegni C, Colafranceschi M, Ottini L, Dogliotti E. Microsatellite instability as a marker of prognosis and response to therapy: a meta-analysis of colorectal cancer survival data. Eur J Cancer 2010;46:2788–2798.

115. Sargent DJ, Marsoni S, Monges G, et al. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. J Clin Oncol 2010;28:3219–3226.

116. Hampel H, Frankel WL, Martin E, et al. Feasibility of screening for Lynch syndrome among patients with colorectal cancer. J Clin Oncol 2008;26:5783–5788.

117. Hampel H, Frankel WL, Martin E, et al. Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). N Engl J Med 2005;352:1851–1860.

118. Umar A, Boland CR, Terdiman JP, et al. Revised Bethesda guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst 2004;96:261–268.

119. Umar A, Risinger JI, Hawk ET, Barrett JC. Testing guidelines for hereditary non-polyposis colorectal cancer. Nat Rev Cancer 2004;4:153–158.

120. Hutchins G, Southward K, Handley K, et al. Value of mismatch repair, KRAS, and BRAF mutations in predicting recurrence and benefits from chemotherapy in colorectal cancer. J Clin Oncol 2011;29:1261–1270.

121. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. J Clin Oncol 2005;23:609–618.

122. Locker GY, Hamilton S, Harris J, et al. ASCO 2006 update of recommendations for the use of tumor markers in gastrointestinal cancer. J Clin Oncol 2006;24:5313–5327.

123. Bertagnolli MM, Niedzwiecki D, Compton CC, et al. Microsatellite instability predicts improved response to adjuvant therapy with irinotecan, fluorouracil, and leucovorin in stage III colon cancer: Cancer and Leukemia Group B protocol 89803. J Clin

Oncol 2009;27:1814–1821.

124. Domingo E, Laiho P, Ollikainen M, et al. BRAF screening as a low-cost effective strategy for simplifying HNPCC genetic testing. J Med Genet 2004;41:664–668.

125. Julie C, Tresallet C, Brouquet A, et al. Identification in daily practice of patients with Lynch syndrome (hereditary nonpolyposis colorectal cancer): revised Bethesda guidelines-based approach versus molecular screening. Am J Gastroenterol 2008;103:2825–2835; quiz 2836.

126. Wanebo HJ, Rao B, Pinsky CM, et al. Preoperative carcinoembryonic antigen level as a prognostic indicator in colorectal cancer. N Engl J Med 1978;299:448–451.

127. Wang JY, Tang R, Chiang JM. Value of carcinoembryonic antigen in the management of colorectal cancer. Dis Colon Rectum 1994;37:272–277.

128. Cohen SJ, Punt CJ, Iannotti N, et al. Prognostic significance of circulating tumor cells in patients with metastatic colorectal cancer. Ann Oncol 2009;20:1223–1229.

129. Rahbari NN, Aigner M, Thorlund K, et al. Meta-analysis shows that detection of circulating tumor cells indicates poor prognosis in patients with colorectal cancer. Gastroenterology 2010;138:1714–1726.

130. Tol J, Koopman M, Miller MC, et al. Circulating tumour cells early predict progression-free and overall survival in advanced colorectal cancer patients treated with chemotherapy and targeted agents. Ann Oncol 2010;21:1006–1012.

131. Rosenberg R, Maak M, Simon I, et al. Independent validation of a prognostic genomic profile (ColoPrint) for stage II colon cancer (CC) patients [abstract]. J Clin Oncol 2011;29(Suppl 1):Abstract 358.

132. Salazar R, Roepman P, Capella G, et al. Gene expression signature to improve prognosis prediction of stage II and III colorectal cancer. J Clin Oncol 2011;29:17–24.

133. Dahlin AM, Palmqvist R, Henriksson ML, et al. The role of the CpG island methylator phenotype in colorectal cancer prognosis depends on microsatellite instability screening status. Clin Cancer Res 2010;16:1845–1855.

134. Barault L, Charon-Barra C, Jooste V, et al. Hypermethylator phenotype in sporadic colon cancer: study on a population-based series of 582 cases. Cancer Res 2008;68:8541–8546.

135. Ogino S, Nosho K, Kirkner GJ, et al. A cohort study of tumoral LINE-1 hypomethylation and prognosis in colon cancer. J Natl Cancer Inst 2008;100:1734–1738.

136. Ahn JB, Chung WB, Maeda O, et al. DNA methylation predicts recurrence from resected stage III proximal colon cancer. Cancer 2011;117:1847–1854.

137. Ogino S, Galon J, Fuchs CS, Dranoff G. Cancer immunology-analysis of host and tumor factors for personalized medicine. Nat Rev Clin Oncol, in press. doi: 10.1038/nrclinonc.2011.122.

138. Dahlin AM, Henriksson ML, Van Guelpen B, et al. Colorectal cancer prognosis depends on T-cell infiltration and molecular characteristics of the tumor. Mod Pathol 2011;24:671–682.

139. Nosho K, Baba Y, Tanaka N, et al. Tumour-infiltrating T-cell subsets, molecular changes in colorectal cancer, and prognosis: cohort study and literature review. J Pathol 2010;222:350–366.

140. Ogino S, Nosho K, Irahara N, et al. Lymphocytic reaction to colorectal cancer is associated with longer survival, independent of lymph node count, microsatellite instability, and CpG island methylator phenotype. Clin Cancer Res 2009;15:6412–6420.

141. Galon J, Costes A, Sanchez-Cabo F, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 2006;313:1960–1964.

142. Mlecnik B, Tosolini M, Kirilovsky A, et al. Histopathologic-based prognostic factors of colorectal cancers are associated with the state of the local immune reaction. J Clin Oncol 2011;29:610–618.

143. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004;350:2129–2139.

144. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004;304:1497–1500.

145. Pao W, Miller V, Zakowski M, et al. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc Natl Acad Sci U S A 2004;101:13306–13311.

146. Riely GJ, Politi KA, Miller VA, Pao W. Update on epidermal growth factor receptor mutations in non-small cell lung cancer. Clin Cancer Res 2006;12:7232–7241.

147. Shigematsu H, Lin L, Takahashi T, et al. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. J Natl Cancer Inst 2005;97:339–346.

148. Ettinger DS, Akerley W, Borghaei H, et al. NCCN Clinical Practice Guidelines in Oncology: Non–Small Cell Lung Cancer. Version 2, 2012. Available at: http://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf. Accessed October 18, 2011.

149. Keedy VL, Temin S, Somerfield MR, et al. American Society of Clinical Oncology provisional clinical opinion: epidermal growth factor receptor (EGFR) mutation testing for patients with advanced non-small-cell lung cancer considering first-line EGFR tyrosine kinase inhibitor therapy. J Clin Oncol 2011;29:2121–2127.

150. Pao W, Ladanyi M. Epidermal growth factor receptor mutation testing in lung cancer: searching for the ideal method. Clin Cancer Res 2007;13:4954–4955.

151. Dacic S. EGFR assays in lung cancer. Adv Anat Pathol 2008;15:241–247.

152. Han SW, Kim TY, Jeon YK, et al. Optimization of patient selection for gefitinib in non-small cell lung cancer by combined analysis of epidermal growth factor receptor mutation, K-ras mutation, and Akt phosphorylation. Clin Cancer Res 2006;12:2538–2544.

153. Sholl LM, Xiao Y, Joshi V, et al. EGFR mutation is a better predictor of response to tyrosine kinase inhibitors in non-small cell lung carcinoma than FISH, CISH, and immunohistochemistry. Am J Clin Pathol 2010;133:922–934.

154. Kosaka T, Yatabe Y, Endoh H, et al. Mutations of the epidermal growth factor receptor gene in lung cancer: biological and clinical implications. Cancer Res 2004;64:8919–8923.

155. Marchetti A, Milella M, Felicioni L, et al. Clinical implications of KRAS mutations in lung cancer patients treated with tyrosine kinase inhibitors: an important role for mutations in minor clones. Neoplasia 2009;11:1084–1092.

156. Shigematsu H, Gazdar AF. Somatic mutations of epidermal growth factor receptor signaling pathway in lung cancers. Int J Cancer 2006;118:257–262.

157. Miller VA, Riely GJ, Zakowski MF, et al. Molecular characteristics of bronchioloalveolar carcinoma and adenocarcinoma, bronchioloalveolar carcinoma subtype, predict response to erlotinib. J Clin Oncol 2008;26:1472–1478.

158. Pao W, Miller VA, Politi KA, et al. Acquired resistance of lung

adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. PLoS Med 2005;2:e73.

159. Pao W, Wang TY, Riely GJ, et al. KRAS mutations and primary resistance of lung adenocarcinomas to gefitinib or erlotinib. PLoS Med 2005;2:e17.

160. Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med 2010;363:1693–1703.

161. Rodig SJ, Mino-Kenudson M, Dacic S, et al. Unique clinicopathologic features characterize ALK-rearranged lung adenocarcinoma in the western population. Clin Cancer Res 2009;15:5216–5223.

162. Rodig SJ, Shapiro GI. Crizotinib, a small-molecule dual inhibitor of the c-Met and ALK receptor tyrosine kinases. Curr Opin Investig Drugs 2010;11:1477–1490.

163. An investigational drug, PF-02341066 is being studied versus standard of care in patients with advanced non-small cell lung cancer with a specific gene profile involving the anaplastic lymphoma kinase (ALK) gene. Available at: http://www.clinicaltrials.gov/show/NCT00932893. Accessed September 30, 2011.

164. FDA approves Xalkori with companion diagnostic for a type of late-stage lung cancer. Available at: http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm269856.htm. Accessed September 30, 2011.

165. Mino-Kenudson M, Chirieac LR, Law K, et al. A novel, highly sensitive antibody allows for the routine detection of ALK-rearranged lung adenocarcinomas by standard immunohistochemistry. Clin Cancer Res 2010;16:1561–1571.

166. Olaussen KA, Dunant A, Fouret P, et al. DNA repair by ERCC1 in non-small-cell lung cancer and cisplatin-based adjuvant chemotherapy. N Engl J Med 2006;355:983–991.

167. Simon GR, Sharma S, Cantor A, et al. ERCC1 expression is a predictor of survival in resected patients with non-small cell lung cancer. Chest 2005;127:978–983.

168. Pao W, Girard N. New driver mutations in non-small-cell lung cancer. Lancet Oncol 2011;12:175–180.

169. Azzoli CG, Baker S, Jr., Temin S, et al. American Society of Clinical Oncology Clinical Practice Guideline update on chemotherapy for stage IV non-small-cell lung cancer. J Clin Oncol 2009;27:6251–6266.

170. Andriole GL, Levin DL, Crawford ED, et al. Prostate Cancer Screening in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial: findings from the initial screening round of a randomized trial. J Natl Cancer Inst 2005;97:433–438.

171. Hugosson J, Carlsson S, Aus G, et al. Mortality results from the Goteborg randomised population-based prostate-cancer screening trial. Lancet Oncol 2010;11:725–732.

172. Schroder FH, Hugosson J, Roobol MJ, et al. Screening and prostate-cancer mortality in a randomized European study. N Engl J Med 2009;360:1320–1328.

173. Carter HB, Ferrucci L, Kettermann A, et al. Detection of lifethreatening prostate cancer with prostate-specific antigen velocity during a window of curability. J Natl Cancer Inst 2006;98:1521–1527.

174. Mohler JL, Armstrong AJ, Bahnson RR, et al. NCCN Clinical Practice Guidelines in Oncology: Prostate Cancer. Version 4, 2011. Available at: http://www.nccn.org/professionals/physician_gls/pdf/prostate.pdf. Accessed October 18, 2011.

175. Freedland SJ, Humphreys EB, Mangold LA, et al. Risk of prostate cancer-specific mortality following biochemical recurrence after radical prostatectomy. JAMA 2005;294:433–439.

176. Pound CR, Partin AW, Eisenberger MA, et al. Natural history of progression after PSA elevation following radical prostatectomy. JAMA 1999;281:1591–1597.

177. Chun FK, de la Taille A, van Poppel H, et al. Prostate cancer gene 3 (PCA3): development and internal validation of a novel biopsy nomogram. Eur Urol 2009;56:659–667.

178. Hessels D, Schalken JA. The use of PCA3 in the diagnosis of prostate cancer. Nat Rev Urol 2009;6:255–261.

179. Roobol MJ, Haese A, Bjartell A. Tumour markers in prostate cancer III: biomarkers in urine. Acta Oncol 2011;50(Suppl 1):85–89.

180. Aubin SM, Reid J, Sarno MJ, et al. PCA3 molecular urine test for predicting repeat prostate biopsy outcome in populations at risk: validation in the placebo arm of the dutasteride REDUCE trial. J Urol 2010;184:1947–1952.

181. de la Taille A, Irani J, Graefen M, et al. Clinical evaluation of the PCA3 assay in guiding initial biopsy decisions. J Urol 2011;185:2119–2125.

182. Scher HI, Heller G, Molina A, et al. Evaluation of circulating tumor cell (CTC) enumeration as an efficacy response biomarker of overall survival (OS) in metastatic castration-resistant prostate cancer (mCRPC): planned final analysis (FA) of COU-AA-301, a randomized, double-blind, placebo-controlled, phase III study of abiraterone acetate (AA) plus low-dose prednisone (P) post docetaxel [abstract]. J Clin Oncol 2011;29(Suppl 1):Abstract LBA4517.

183. Berger MF, Lawrence MS, Demichelis F, et al. The genomic complexity of primary human prostate cancer. Nature 2011;470:214–220.

184. Mackinnon AC, Yan BC, Joseph LJ, Al-Ahmadie HA. Molecular biology underlying the clinical heterogeneity of prostate cancer: an update. Arch Pathol Lab Med 2009;133:1033–1040.

185. Dohner K, Dohner H. Molecular characterization of acute myeloid leukemia. Haematologica 2008;93:976–982.

186. Mrozek K, Heerema NA, Bloomfield CD. Cytogenetics in acute leukemia. Blood Rev 2004;18:115–136.

187. Mardis ER, Ding L, Dooling DJ, et al. Recurring mutations found by sequencing an acute myeloid leukemia genome. N Engl J Med 2009;361:1058–1066.

188. Mrozek K, Marcucci G, Paschka P, et al. Clinical relevance of mutations and gene-expression changes in adult acute myeloid leukemia with normal cytogenetics: are we ready for a prognostically prioritized molecular classification? Blood 2007;109:431–448.

189. Swerdlow SH, Campo E, Harris NL, et al., eds. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon, France: IARC Press; 2008.

190. O'Donnell MR, Abboud CN, Altman J, et al. NCCN Clinical Practice Guidelines in Oncology: Acute Myeloid Leukemia. Version 2, 2011. Available at: http://www.nccn.org/professionals/physician_gls/pdf/aml.pdf. Accessed October 18, 2011.

191. Dohner H, Estey EH, Amadori S, et al. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. Blood 2010;115:453–474.

192. Falini B, Mecucci C, Tiacci E, et al. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. N Engl J Med 2005;352:254–266.

193. Schlenk RF, Dohner K, Krauter J, et al. Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. N Engl J Med 2008;358:1909–1918.

194. Becker H, Marcucci G, Maharry K, et al. Favorable prognostic

impact of NPM1 mutations in older patients with cytogenetically normal de novo acute myeloid leukemia and associated gene- and microRNA-expression signatures: a Cancer and Leukemia Group B study. J Clin Oncol 2010;28:596–604.

195. Koschmieder S, Halmos B, Levantini E, Tenen DG. Dysregulation of the C/EBPalpha differentiation pathway in human cancer. J Clin Oncol 2009;27:619–628.

196. Burnett AK, Hills RK, Green C, et al. The impact on outcome of the addition of all-trans retinoic acid to intensive chemotherapy in younger patients with nonacute promyelocytic acute myeloid leukemia: overall results and results in genotypic subgroups defined by mutations in NPM1, FLT3, and CEBPA. Blood 2010;115:948–956.

197. Marcucci G, Maharry K, Radmacher MD, et al. Prognostic significance of, and gene and microRNA expression signatures associated with, CEBPA mutations in cytogenetically normal acute myeloid leukemia with high-risk molecular features: a Cancer and Leukemia Group B study. J Clin Oncol 2008;26:5078–5087.

198. Taskesen E, Bullinger L, Corbacioglu A, et al. Prognostic impact, concurrent genetic mutations, and gene expression features of AML with CEBPA mutations in a cohort of 1182 cytogenetically normal AML patients: further evidence for CEBPA double mutant AML as a distinctive disease entity. Blood 2011;117:2469–2475.

199. Wouters BJ, Lowenberg B, Erpelinck-Verschueren CA, et al. Double CEBPA mutations, but not single CEBPA mutations, define a subgroup of acute myeloid leukemia with a distinctive gene expression profile that is uniquely associated with a favorable outcome. Blood 2009;113:3088–3091.

200. Paschka P. Core binding factor acute myeloid leukemia. Semin Oncol 2008;35:410–417.

201. Paschka P, Marcucci G, Ruppert AS, et al. Adverse prognostic significance of KIT mutations in adult acute myeloid leukemia with inv(16) and t(8;21): a Cancer and Leukemia Group B study. J Clin Oncol 2006;24:3904–3911.

202. Whitman SP, Ruppert AS, Marcucci G, et al. Long-term disease-free survivors with cytogenetically normal acute myeloid leukemia and MLL partial tandem duplication: a Cancer and Leukemia Group B study. Blood 2007;109:5164–5167.

203. Tang JL, Hou HA, Chen CY, et al. AML1/RUNX1 mutations in 470 adult patients with de novo acute myeloid leukemia: prognostic implication and interaction with other gene alterations. Blood 2009;114:5352–5361.

204. Gaidzik VI, Schlenk RF, Moschny S, et al. Prognostic impact of WT1 mutations in cytogenetically normal acute myeloid leukemia: a study of the German-Austrian AML Study Group. Blood 2009;113:4505–4511.

205. Paschka P, Marcucci G, Ruppert AS, et al. Wilms' tumor 1 gene mutations independently predict poor outcome in adults with cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study. J Clin Oncol 2008;26:4595–4602.

206. Virappane P, Gale R, Hills R, et al. Mutation of the Wilms' tumor 1 gene is a poor prognostic factor associated with chemotherapy resistance in normal karyotype acute myeloid leukemia: the United Kingdom Medical Research Council Adult Leukaemia Working Party. J Clin Oncol 2008;26:5429–5435.

207. Marcucci G, Maharry K, Wu YZ, et al. IDH1 and IDH2 gene mutations identify novel molecular subsets within de novo cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study. J Clin Oncol 2010;28:2348–2355.

208. Paschka P, Schlenk RF, Gaidzik VI, et al. IDH1 and IDH2 mutations are frequent genetic alterations in acute myeloid leukemia and confer adverse prognosis in cytogenetically normal acute myeloid leukemia with NPM1 mutation without FLT3 internal tandem duplication. J Clin Oncol 2010;28:3636–3643.

209. Carbuccia N, Trouplin V, Gelsi-Boyer V, et al. Mutual exclusion of ASXL1 and NPM1 mutations in a series of acute myeloid leukemias. Leukemia 2010;24:469–473.

210. Haferlach C, Dicker F, Herholz H, et al. Mutations of the TP53 gene in acute myeloid leukemia are strongly associated with a complex aberrant karyotype. Leukemia 2008;22:1539–1541.

211. Ley TJ, Ding L, Walter MJ, et al. DNMT3A mutations in acute myeloid leukemia. N Engl J Med 2010;363:2424–2433.

212. Metzeler KH, Maharry K, Radmacher MD, et al. TET2 mutations improve the new European LeukemiaNet risk classification of acute myeloid leukemia: a Cancer and Leukemia Group B study. J Clin Oncol 2011;29:1373–1381.

213. Neubauer A, Maharry K, Mrozek K, et al. Patients with acute myeloid leukemia and RAS mutations benefit most from postremission high-dose cytarabine: a Cancer and Leukemia Group B study. J Clin Oncol 2008;26:4603–4609.

214. Nibourel O, Kosmider O, Cheok M, et al. Incidence and prognostic value of TET2 alterations in de novo acute myeloid leukemia achieving complete remission. Blood 2010;116:1132–1135.

215. Gilbert MR, Wang M, Aldape KD, et al. RTOG 0525: a randomized phase III trial comparing standard adjuvant temozolomide (TMZ) with a dose-dense (dd) schedule in newly diagnosed glioblastoma (GBM) [abstract]. J Clin Oncol 2011;29(Suppl 1):Abstract 2006.

216. Jeuken JW, Wesseling P. MAPK pathway activation through BRAF gene fusion in pilocytic astrocytomas; a novel oncogenic fusion gene with diagnostic, prognostic, and therapeutic potential. J Pathol 2010;222:324–328.

217. Straver ME, Glas AM, Hannemann J, et al. The 70-gene signature as a response predictor for neoadjuvant chemotherapy in breast cancer. Breast Cancer Res Treat 2010;119:551–558.

218. Knauer M, Mook S, Rutgers EJ, et al. The predictive value of the 70-gene signature for adjuvant chemotherapy in early breast cancer. Breast Cancer Res Treat 2010;120:655–661.

219. Budd GT, Cristofanilli M, Ellis MJ, et al. Circulating tumor cells versus imaging—predicting overall survival in metastatic breast cancer. Clin Cancer Res 2006;12:6403–6409.

220. Cristofanilli M, Hayes DF, Budd GT, et al. Circulating tumor cells: a novel prognostic factor for newly diagnosed metastatic breast cancer. J Clin Oncol 2005;23:1420–1430.

221. De Giorgi U, Valero V, Rohren E, et al. Circulating tumor cells and [18F]fluorodeoxyglucose positron emission tomography/computed tomography for outcome prediction in metastatic breast cancer. J Clin Oncol 2009;27:3303–3311.

222. Tol J, Dijkstra JR, Klomp M, et al. Markers for EGFR pathway activation as predictor of outcome in metastatic colorectal cancer patients treated with or without cetuximab. Eur J Cancer 2010;46:1997–2009.

223. Douillard JY, Shepherd FA, Hirsh V, et al. Molecular predictors of outcome with gefitinib and docetaxel in previously treated non-small-cell lung cancer: data from the randomized phase III INTEREST trial. J Clin Oncol 2010;28:744–752.

224. Kerr D, Gray R, Quirke P, et al. A quantitative multigene RT-PCR assay for prediction of recurrence in stage II colon cancer: selection of the genes in four large studies and results of the independent, prospectively designed QUASAR validation study [abstract]. J Clin Oncol 2009;27(Suppl 1):Abstract 4000.

225. Venook AP, Niedzwiecki D, Lopatin M, et al. Validation of a 12-gene colon cancer recurrence score (RS) in patients (pts) with stage II colon cancer (CC) from CALGB 9581 [abstract]. J Clin Oncol 2011;29(Suppl 1):Abstract 3518.

226. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947–957.

227. Yang CH, Fukuoka M, Mok TS, et al. Final overall survival (OS) results from a phase III, randomised, open-label, first-line study of gefitinib (G) v carboplatin/paclitaxel (C/P) in clinically selected patients with advanced nonsmall cell lung cancer (NSCLC) in Asia (IPASS). Ann Oncol 2010;21:viii1–viii2.

228. Fukuoka M, Wu YL, Thongprasert S, et al. Biomarker analyses and final overall survival results from a phase III, randomized, open-label, first-line study of gefitinib versus carboplatin/paclitaxel in clinically selected patients with advanced non-small-cell lung cancer in Asia (IPASS). J Clin Oncol 2011;29:2866–2874.

229. Lee DH, Park K, Kim JH, et al. Randomized phase III trial of gefitinib versus docetaxel in non-small cell lung cancer patients who have previously received platinum-based chemotherapy. Clin Cancer Res 2010;16:1307–1314.

230. Maemondo M, Inoue A, Kobayashi K, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med 2010;362:2380–2388.

231. Mitsudomi T, Steinberg SM, Oie HK, et al. ras gene mutations in non-small cell lung cancers are associated with shortened survival irrespective of treatment intent. Cancer Res 1991;51:4999–5002.

232. Linardou H, Dahabreh IJ, Kanaloupiti D, et al. Assessment of somatic k-RAS mutations as a mechanism associated with resistance to EGFR-targeted agents: a systematic review and meta-analysis of studies in advanced non-small-cell lung cancer and metastatic colorectal cancer. Lancet Oncol 2008;9:962–972.

233. Mao C, Qiu LX, Liao RY, et al. KRAS mutations and resistance to EGFR-TKIs treatment in patients with non-small cell lung cancer: a meta-analysis of 22 studies. Lung Cancer 2010;69:272–278.

234. Freedland SJ, Partin AW. Detecting prostate cancer with molecular markers: uPM3. Rev Urol 2005;7:236–238.

235. Nogueira L, Corradi R, Eastham JA. Other biomarkers for detecting prostate cancer. BJU Int 2010;105:166–169.

236. de Bono JS, Scher HI, Montgomery RB, et al. Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. Clin Cancer Res 2008;14:6302–6309.

237. Danila DC, Heller G, Gignac GA, et al. Circulating tumor cell number and prognosis in progressive castration-resistant prostate cancer. Clin Cancer Res 2007;13:7053–7058.

Individual Disclosures for NCCN Task Force: Evaluating the Clinical Utility of Tumor Markers in Oncology Panel Members

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Marian Birkeland, PhD	None	None	None	GlaxoSmithKline	5/7/11
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Angelo M. De Marzo, MD, PhD	None	None	None	None	5/12/11
M. Elizabeth Hammond, MD	None	None	None	None	5/7/11
Daniel F. Hayes, MD	GlaxoSmithKline; Pfizer Inc.; Novartis AG; and Veridex, LLC	Chugai Pharmaceutical Co., Ltd; and BioMarker Strategies	Immunicon Corporation; University of Michigan; and Oncimmune LLC	None	5/10/11
A. John Iafrate, MD, PhD	None	Pfizer Inc.; and Abbott Laboratories	None	None	5/10/11
R. Kate Kelley, MD	Genomic Health, Inc.	None	None	None	5/11/11
Guido Marcucci, MD	None	None	None	None	5/12/11
Shuji Ogino, MD, PhD, MS	None	None	None	None	5/13/11
William Pao, MD, PhD	Enzon Pharmaceuticals, Inc.; AstraZeneca Pharmaceuticals LP; and Xcovery	Clovis Oncology; Bristol-Myers Squibb Company; AstraZeneca Pharmaceuticals LP; MolecularMD; and Symphony Evolution, Inc.	MolecularMD	None	5/13/11
Dennis C. Sgroi, MD	None	None	bioTheranostics, Inc.	None	5/12/11

Post-test

1. In order for molecular test results to be used to determine patient care, the test must be:
   a. FDA-approved
   b. FDA-cleared
   c. Performed in a CLIA laboratory
   d. Any of the above
   e. All of the above

2. If a given test is approved or cleared by the FDA, this indicates that it has proven clinical utility. Is this statement true or false?
   a. True
   b. False

3. What is detected by the companion diagnostic recently FDA approved with vemurafenib for treatment of metastatic melanoma?
   a. *ALK* gene fusion
   b. *ESR1* overexpression
   c. *BRAF* V600 mutation
   d. *KRAS* mutation
   e. Serum PSA

4. A hypothetical test for determining somatic mutation in a newly identified gene has been shown to predict response to a new chemotherapy agent. If use of the test is required for prescription of the drug, and both are approved by the FDA, the test would be called a:
   a. Laboratory-developed test (LDT)
   b. CLIA-certified test
   c. Companion diagnostic
   d. CAP-certified test

5. Which of the following must have proven clinical validity?
   a. An LDT performed in a CLIA laboratory
   b. An FDA-cleared test
   c. An FDA-approved test
   d. A molecular test performed in a research laboratory
   e. a and b
   f. b and c
   g. a, b, c, and d
   h. a and d
   i. None of the above

6. The Clinical Laboratory Improvement Amendment (CLIA) of 1988 is administered by:
   a. FDA
   b. CMS
   c. CAP
   d. NCCN

7. What can be done to help prove clinical utility of a molecular test?
   a. Demonstrate that test performance is reproducible in 3 or more clinical laboratories.
   b. Demonstrate that test results predict response/lack of response to a given therapy.
   c. Demonstrate that test results show which subset of patients have the longest progression-free survival.
   d. Demonstrate that test results are statistically significant.

8. Which molecular test is predictive for response to trastuzumab?
   a. HER2*(ERBB2)* protein expression by immunohistochemistry
   b. ER-α*(ESR1)*/PgR(PR) protein expression by immunohistochemistry
   c. HER2*(ERBB2)* amplification by FISH
   d. a and b
   e. a and c
   f. a, b, and c

9. Which tests have proven clinical utility in NSCLC?
   a. *EGFR* mutation and *KRAS* mutation
   b. ERCC1 expression and *BRAF* mutation
   c. *ALK* gene fusion and *EGFR* mutation
   d. *ALK* gene fusion and *KRAS* mutation

10. Which molecular tests have proven clinical utility and outperform Gleason sum as a prognostic for localized prostate cancer?
    a. Baseline PSA level
    b. Urine PCA3 testing
    c. Circulating tumor cell analysis
    d. Gene expression analysis
    e. All of the above
    f. None of the above

11. *IDH1* and *IDH2* mutations have been detected in what types of cancers:
    a. AML
    b. NSCLC
    c. Glioma
    d. a and b
    e. a and c
    f. b and c
    g. None of the above

12. Which methods of testing have proven analytic validity for MSI/MMR assessment in colon cancer?
    a. PCR for MSI
    b. Flow cytometry for MSI
    c. Immunohistochemistry for MMR
    d. FISH for MMR
    e. a and b
    f. a and c
    g. b and c
    h. c and d

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To receive credit, participants will read all portions of this monograph, including all tables, figures, and references. To receive your continuing education credit and certificate, visit http://www.cvent.com/d/rcq8rk to complete the post-test and evaluation.

All post-test scores must be received by midnight on November 18 2012, in order to be eligible for credit.

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