Cigna Medical Coverage Policy

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Subject **Tumor Markers for Cancer**

Table of Contents

Hyperlink to Related Coverage Policies

Assays of Genetic Expression in Tumor Tissue as a Prognosis in Patients with Breast Cancer
Circulating Tumor Cells Testing
Gene-Based Testing for Prostate Cancer Screening, Detection and Disease Monitoring
Pharmacogenetic Testing
Prostate-Specific Antigen (PSA) Screening for Prostate Cancer
Topographic Genotyping (PathFinderTG® Test)
Tumor In Vitro Chemosensitivity and Chemoresistance Assays
Oncology Medications

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Coverage Policy

Tumor Markers for Cancer

Cigna covers EACH of the following tumor markers as medically necessary for the diagnosis and management of the specific condition(s) noted:

Tumor Marker	Condition(s)
AFP (alpha-fetoprotein)	• Primary hepatocellular cancer
AFP in combination with b-HCG (beta-human chorionic gonadotropin)	• Nonseminoma germ cell testicular cancer • Germ cell ovarian cancer • Undiagnosed pelvic mass
B_2M (beta$_2$-microglobulin)	• Multiple myeloma
Bladder-tumor associated antigen (BTA)	• Bladder cancer
Calcitonin	• Thyroid medullary carcinoma
CA (cancer antigen) 15-3, CA 27.29, BR 27.29 or Truquant RIA	• Metastatic breast cancer

CA 19.9	• Pancreatic cancer
CA 125	• Epithelial ovarian cancer • Endometrial cancer • Undiagnosed pelvic mass
CEA (carcinoembryonic antigen)	• Colorectal cancer • Medullary thyroid cancer • Metastatic breast cancer
CgA (chromogranin A)	• Neuroendocrine tumors (e.g., carcinoid tumors, neuroblastoma, and small cell lung cancer)
C-kit (CD-117 [cluster of differentiation-117])	• Gastrointestinal stromal tumors
ER/PR (estrogen receptors and progesterone receptors)	• Breast cancer
5-HIAA (5-hydrocyindoleascetic acid)	• Carcinoid tumors
Gene expression classifier for thyroid nodule (i.e., Afirma® Thyroid FNA Analysis)	• Cytologically indeterminate thyroid nodule
HCG (human chorionic gonadotropin)	• Trophoblastic testicular cancer • Trophoblastic ovarian cancer
HER2 (human epidermal growth factor receptor 2) when performed by immunohistochemical (IHC) and/or fluorescent in situ hybridization (FISH)	• Breast cancer • Gastric or esophagogastric junction adenocarcinoma (i.e., inoperable, locally advanced, recurrent or metastatic disease in an individual for whom trastuzumab therapy is being considered and individual has had no prior treatment for metastatic disease).
ImmunoCyte™/yCyte+™	• Bladder cancer
MPO (myeloperoxidase)	• Acute myeloid leukemia
Nuclear-Matrix Protein (NMP22)	• Bladder cancer
NSE (neuron-specific enolase)	• Small cell lung cancer
PSA (prostate-specific antigen)	• Prostate cancer (For Screening - Refer to the Prostate-Specific Antigen (PSA) Screening for Prostate Cancer Coverage Policy)
Thyroglobulin	• Differentiated thyroid cancer
UroVysion™	• Bladder cancer

Cigna does not cover any of the tumor markers listed above for ANY cancer indication not otherwise listed as covered because it is considered experimental, investigational, unproven.

Cigna does not cover ANY of the following tumor markers for the screening, staging, diagnosis, monitoring and/or surveillance of a cancer because EACH is considered experimental, investigational or unproven (this list may not be all-inclusive):

- autoantibody panel for lung cancer (e.g., EarlyCDT™)
- CA 50 (cancer antigen 50)
- CA 72-4 (cancer antigen 72-4)
- CA 195 (cancer antigen 195)
- CA 242 (cancer antigen 242)
- CA 549 (cancer antigen 549)
- CAM 17.1 (monoclonal antimucin antibody 17.1)
- Cathepsin-D (Ab-1 monoclonal antibody)
- CYFRA21-1 (cytokeratin fragment 19)
- DCP (des-gamma-carboxy-prothrombin)
- DNA Ploidy (deoxyribonucleic acid ploidy)
- DU-PAN-2 (sialylated carbohydrate antigen)
- GCC (guanylyl cyclase C)
- gene mutation marker panels for indeterminate thyroid nodule diagnosis (e.g., miRInform)

- HER2 gene amplification testing of breast cancer tissue (e.g., SPoT-Light® HER2 CISH™)
- hMAM (human mammoglobulin)
- LASA-P (lipid-associated sialic acid in plasma)
- LPA (lysophosphatidic acid)
- MCA (mucin-like cancer antigen)
- MCAM (melanoma cell adhesion molecule)
- methylation analysis of DNA for determining tumor grade (e.g., DecisionDX-G-CIMP)
- microarray analysis for measuring the degree of similarity in undifferentiated tumor types (e.g., Pathwork® Tissue of Origin)
- microRNA testing for ANY of the following indications:
  - differentiating squamous cell non-small cell lung cancer (e.g., ProOnc SquamousDx™)
  - aiding in the diagnosis of mesothelioma (e.g., ProOnc MesotheliomaDx™)
  - identifying the tissue of origin of a metastatic tumor (e.g., ProOnc TumorSourceDx™)
- multigene expression testing for ANY of the following:
  - colon cancer recurrence (e.g., Oncotype DX® Colon Cancer Assay)
  - determining the molecular signature of a glioblastoma multiforme (GBM) tumor (e.g., DecisionDx-GBM)
  - determining the molecular signature of a uveal melanoma (UM) tumor (e.g., DecisionDX-UM)
  - prediction of breast cancer occurrence (e.g., OncoVue® Breast Cancer Risk Test)
  - prostate cancer (e.g., Oncotype DX Prostate)
- multiprotein panel testing for detection of ovarian cancer in a pelvic mass (e.g., OVA1™, ROMA™)
- OPN (osteopontin)
- P53 (monoclonal antibody)
- P-LAP (placental alkaline phosphatase)
- PSMA (prostate-specific membrane antigen)
- S 100
- SCC-Ag (squamous cell carcinoma antigen)
- SLEX (sialyl Lewis x-antigen)
- SLX (sialyl X)
- systems pathology for predicting risk of recurrence in prostate cancer (e.g., Prostate PX+, Post-Op PX®**)**
- TA-90
- TATI (tumor-associated trypsin inhibitor)
- TNF-a (tumor necrosis factor alpha)
- TPA (tissue polypeptide antigen)
- tumor profiling (e.g., Caris Target Now™)

Paraneoplastic Antibodies

Cigna covers the following paraneoplastic (onconeural) antibodies as medically necessary for the evaluation of neurological symptoms when the diagnosis remains uncertain following conventional work-up and an occult neoplasm is suspected:

- anti-Hu (ANNA-1 [antineuronal nuclear autoantibodies-1])
- anti-Yo (PCA-1 [Purkinje cell antibody-1])
- anti-CV2 (CRMP5 [collapsing mediator response protein5])
- anti-Ri (ANNA-2)
- anti-MA2 (Ta)
- anti-amphiphysin

General Background

Tumor markers are substances produced by cancer or other cells in the body in response to cancer, or certain benign conditions. Most tumor markers are proteins but may also be patterns of gene expression and changes to DNA. Tumor markers are made by normal cells but are produced at a much higher level in the presence of a cancer. Tumor markers may be found in the blood, plasma, other bodily fluids (e.g., urine, saliva, sputum,

cerebrospinal fluid, or effusions) and/or tissue. Although an abnormal tumor marker level may suggest or cancer, their presence alone does not confirm a diagnosis. Tumor markers are typically combined with other diagnostic studies (e.g., laboratory test, biopsy, radiological imaging) to confirm the diagnosis. These markers may not be elevated in the presence of some diseases or cancers, especially in early stages of the disease, may not be specific to a particular type of disease or cancer, and/or may be elevated by more than one type of disease or cancer.

In some types of cancers, tumor marker levels may reflect the extent or stage of the disease and can be useful in determining the most effective treatment and how well the disease will respond to the treatment. Typically, the primary use of tumor markers is to monitor a cancer's response to treatment with periodic measurements following therapy. Following therapy, a decrease in the marker level may indicate a response to therapy as opposed to consistently elevated or rising marker levels which may be indicative of a lack of response to treatment or recurrence of the disease. The evidence in the published peer-reviewed literature and professional societies support tumor makers for the diagnosis and management of some cancers, while other tumor markers are still evolving and their clinical utility has not been proven.

U.S. Food and Drug Administration (FDA)
Devices with reagents that are used to "qualitatively or quantitatively measure, by immunochemical techniques, tumor-associated antigens in serum, plasma, urine, or other body fluids" and intended as an aid in monitoring patients for disease progress or response to therapy or for the detection of recurrent or residual disease" are approved by the FDA 510(k) process (FDA, 2009). Examples of these devices include the ARCHITECT® CA 125 II™ Assay (Fujirebio Diagnostics, Inc., Malvern, PA) and the IMMULITE® 2000 Calcitonin (Diagnostic Products Corporation, Los Angeles, CA) (FDA, 2004; FDA, 2002).

Urine-based tumor markers used for the management of bladder cancer also require FDA approval under the 510(k) process or the premarket approval process. The tests are used to aide in the diagnosis and monitoring of bladder cancer, are not stand alone tests and are to be used in conjunction with cystoscopy, the gold standard for detecting bladder cancer. Examples of these urine-based tests include: BTA stat® Test (Bard Diagnostic, Redmond, WA); ImmunoCyte™ (Diagno-Cure Inc., Saite-Foy, Quebec, Canada); NMP22® BladderChek® Test (Matritech, Newton, MA); and the UroVysion™ Bladder Cancer Kit (UroVysion Kit, Vysis, Inc. [a wholly-owned subsidiary of Abbott Laboratories] Downers Grove, IL) (FDA, 2005; FDA, 2000).

Specific Tumor Markers (Refer to Appendix A for specific tumor markers by cancer type)
Evidence in the published peer-reviewed literature and/or professional societies and organizations, support the following tumor makers as established markers for the screening, staging, diagnosing, treatment planning, and/or follow-up of the indicated carcinomas. By using the information that these markers provide, patient-specific treatment protocols may be developed, implemented, and/or monitored for improved outcomes.

- AFP (alpha-fetaprotein) is recommended for the management of primary liver cancer or primary hepatocellular carcinoma (HCC) (also called hepatoma).
- AFP in combination with b-HCG (beta- human chorionic gonadotropin) is indicated for the diagnostic work-up, treatment monitoring and/or follow-up of individuals with suspected nonseminoma testicular germ cell carcinoma, germ cell ovarian cancer or an undiagnosed pelvic mass.
- β_2M (beta$_2$–microglobulin) is included in the initial diagnostic work-up for multiple myeloma and is useful in the staging of the disease.
- Calcitonin may be used to help diagnose early thyroid medullary carcinoma and is recommended to assist in determining the extent of surgical intervention and follow-up of residual disease.
- CA (cancer antigen) 15-3 also referred to as CA 27.29, BR 27.29 or Truquant RIA, is an established marker used for the monitoring and follow-up of breast cancer.
- CA 19.9 (carbohydrate antigen 19.9) is the standard tumor marker for pancreatic cancer. It is also expressed in other malignancies (e.g., colorectal, lung, liver, gallbladder and gastric) but its usefulness in other cancers has not been proven. The 2012 practice parameters by the American Society of Colon & Rectal Surgeons (ASCRS) stated that, other than CEA, there is insufficient evidence to support the routine use of tumor markers such as CA19-9 in the routine evaluation of patients with colon cancer.
- CA-125 is the standard tumor marker used for treatment monitoring and follow-up of epithelial ovarian cancer, endometrial cancer, and undiagnosed suspicious pelvic masses. CA-125 levels may also be

elevated in cancers of the pancreas, liver, colon, breast, lung, and digestive tract, but its clinical utility in these cancers has not been established.

- CEA (carcinoembryonic antigen) is used for the management of colorectal cancer (CRC), medullary thyroid cancer and metastatic breast cancer. CEA has a low sensitivity and specificity; therefore, it not recommended as a screening tool.
- CgA (chromogranin A) is used primarily in the diagnosis and monitoring of patients with carcinoid tumors, islet cell tumors, pheochromocytoma, neuroblastoma, and other neuroendocrine tumors.
- C-kit, KIT, or CD-117 (cluster of differentiation-117) is a gene found in all cells of the body and leads to the formation of a protein called KIT. Most gastrointestinal stromal tumors (GIST) contain c-kit, making the test a useful diagnostic tool for this cancer. According to the National Comprehensive Cancer Network®, (NCCN®) (2010), 95% of GISTs are positive for c-kit. C-kit is used to determine a patient's eligibility for treatment with Gleevec® (imatinib mesylate), a protein-tyrosine kinase inhibitor. Gleevec inhibits c-kit inducing apoptosis. Gleevec is also indicated for the treatment of other cancers (e.g., chronic myeloid leukemia) by a proposed mechanism of inhibiting the BCR-ABL gene.
- ER/PR (estrogen receptors/progesterone receptors) receptors are recommended as part of the general work-up and treatment planning in breast cancer patients. These receptors are used to predict response to therapy and the likelihood of recurrence.
- 5-Hydroxyindoleascetic Acid (5-HIAA) is an established marker for use in the diagnosis of carcinoid tumors
- Gene expression classifier for cytologically indeterminate thyroid nodule: Afirma® Thyroid FNA Analysis (Veracyte, Inc., San Francisco, CA) is a gene expression classifier test which combines cytopathology by Thyroid Cytopathology Partners (TCP) with Afirma's Gene Expression Classifier (GEC). If the cytopathology sample is indeterminate, GEC is performed. The algorithm uses the expression of 167 genes to classify the specimen including 142 genes in the main classifier (benign or suspicious) and 25 genes that initially filter out rare neoplasms. Veracyte is a CLIA-certified laboratory (Alexander, et al., 2012; Veracyte, 2012).

  In a multicenter retrospective review, Alexander et al. (2013) reported the first analysis of Afirma in a live, clinical environment (n=339). Patients had cytologically indeterminate thyroid nodules and underwent Afirma testing. A total of 165 patients had an atypical or follicular lesion of undetermined significance (AUS/FLUS), 161 had a follicular neoplasm (FN) and 13 nodules were suspicious. Afirma reported 174 benign nodules and 148 suspicious nodules. Four Afirma benign patients vs. 141 suspicious patients were recommended for surgery ($p<0.01$). Of the 121 cytologically indeterminate/Afirma suspicious nodules removed, 53 were malignant. Variability in site-to-site Afirma results varied up to 29% for benign lesions ($p=0.58$) and 47% in cytologically indeterminate/Afirma suspicious lesions ($p=0.11$). An average follow-up of 8.5 months was reported in 71 benign nodules and one nodule proved cancerous. Assuming that surgery would have been performed on all cytologically indeterminate nodules, Afirma significantly decreased the number of patients who underwent surgery. The authors stressed that neither cytological nor Afirma results alone should mandate recommendations for care. Limitations of the study include the retrospective study design, the variability in results from site-to-site (nonsignificant), and lack of follow-up data on 59% of patients with benign Afirma results.

  Alexander et al. (2012) conducted a 19-month prospective, noninterventional, 49-center validation study of 4812 FNAs (n=3789 patients) from thyroid nodules ≥ 1 centimeter, evaluated by fine-needle aspiration (FNA). A total of 577 cytological indeterminate specimens were found, of which 413 had corresponding histopathological specimens from excised lesions. Four specimens were excluded because patient age was less than 21 years. Afirma Thyroid FNA Analysis was used to test 265 indeterminate nodules. Midway through the study the protocol for sample retrieval (2–5 insertions per nodule to two insertions per nodule) and shipment was modified. After FNA, cytologic reports were collected and "reports without a definitive benign or malignant local diagnosis were reviewed by three expert cytopathologists, who reclassified each report according to three categories of the Bethesda System for Reporting Thyroid Cytopathology: atypia (or follicular lesion) of undetermined significance, follicular neoplasm or lesion suspicious for follicular neoplasm, and lesion suspicious for malignancy". Thyroid surgery was performed based on clinical judgment, then histopathological reports and slides were collected and biopsied nodules were matched to resected nodules. All slides were deidentified. Forty-six samples that did not meet criteria were excluded from the study. A total of 85 nodules were classified as malignant yielding a sensitivity of 92% and specificity of 52%. The negative predictive value was 95% for "atypia (or follicular lesion) of undetermined clinical significance", 94% for "follicular neoplasm or lesion suspicious for follicular neoplasm," and 85% for

"suspicious cytologic findings". Author-noted limitations of the study included: 14% of independent classifications were discordant" and "this imperfect interobserver agreement may have affected the sensitivity or specificity of the classifier"; "five of six false negative results for papillary thyroid carcinoma occurred in samples for which the classifier failed to show independent molecular signatures of papillary thyroid carcinoma and follicular content" suggesting that improper sampling technique or heterogeneity of the nodule may contribute to inaccurate results; and the prevalence of cancer in a study (e.g., 32% in this study) affects the negative predictive value. The authors noted that although the study showed that Afirma can identify a subpopulation of patients with a low likelihood of cancer, each clinical decision must be individualized.

The 2013 National Comprehensive Cancer Network® (NCCN) guidelines on thyroid cancer, and the NCCN Biomarker Compendium™ (2012) rate molecular testing for a subset of thyroid cancer as a category 2A (based upon lower-level evidence, there is uniform consensus that the intervention is appropriate). According to NCCN molecular testing to detect individual mutations (e.g., BRAF, RET/PTC, RAS, PAX8/PPAR [peroxisome proliferatory-activated receptors] gamma) or pattern recognition approaches using molecular classifiers may be useful in the evaluation of indeterminate FNA samples. The NCCN recommendations include molecular diagnostics for evaluating 1) FNA results that are suspicious for Follicular or Hurthle cell neoplasms or 2) follicular lesion of undetermined significance. Results of molecular testing may allow for observation of the tumor as opposed to immediate surgical resection if the application of the test results in a predicted risk of malignancy that is comparable to the rate seen in cytologically benign thyroid FNAs (approximately ≤ 5%). Due to the lack of clinical trials involving pediatric patients, this recommendation only applies to adults.

The American Thyroid Association (ATA) Clinical Affairs Committee (Hodak and Rosenthal, 2013) published an official statement to provide direction for clinicians and patients regarding the current state of thyroid molecular diagnosis including Afirma, miRInform and Cleveland Clinic TSHR mRNA Assay. ATA stated that the commercial and noncommercial use of BRAF, RAS, RET/PTC, and PAX8/PPARγ testing have promising roles, but experience with these tests is limited and "no test has perfect sensitivity and specificity". ATA stated that until expert consensus review of existing data is completed, no evidence-based recommendation for or against the use of these tests can be made. They advised clinicians to use caution and to remain cognizant of the limited available data. "Until evidence-based recommendations are available, determining whether or not the limited data available support the use of these methods should be considered on a case-by-case basis".

- HCG (human chorionic gonadotropin) or beta-HCG (β-HCG) may be elevated in patients with trophoblastic testicular cancer or trophoblastic ovarian cancer. HCG can be used to assist in the diagnosis of the cancer and monitoring the effectiveness of treatment.
- HER2 (human epidermal growth factor receptor 2), HER-2/neu, erbB-2 (v-erb-b2 erythroblastic leukemia viral oncogene homolog 2) or EGFR2 (epidermal growth factor receptor), is a protein used to assist in the personal stratification of breast cancer patients to anti-HER-2-based therapies. The marker is also recommended for patients with inoperable locally advanced, recurrent or metastatic gastric or gastroesophageal junction adenocarcinoma in an individual for whom trastuzumab therapy is being considered and the individual has had no prior treatment for metastatic disease (ACS, 2012; NCCN, 2012; Chua et al., 2011; MacKenzie, et al., 2011; Gravalos and Jimeno, 2008).

  The measurement of HER-2 is recommended in primary breast tumors at the time of diagnosis or at the time of recurrence. According to the National Comprehensive Cancer Network® (2012) and the National Academy of Clinical Biochemistry (NACB) (2009), immunohistochemical (IHC) or fluorescent in situ hybridization (FISH) are the two established platforms used to evaluate HER2 levels in breast cancer patients. IHC measures the levels of the HER2 proteins on the surface of the tumor cells using monoclonal or polyclonal antibodies. The antibodies bind to the HER2 protein making it visible under a microscope. Fluorescent in situ hybridization (FISH) is a technique that uses fluorescent pieces of DNA that bind to the HER2 gene causing the gene to light up and be visualized under a microscope. Fish counts the HER2 gene copies and quantifies them in a ratio with chromosome 17 (CEP17). Typically, IHC is the first test used. If the results are unequivocal, or intermediate, FISH may be conducted. If FISH is unequivocal, the test should be repeated or additional cells counted (NACB, 2009). Detailed testing guidelines for IHC and FISH have been published by the American Society of Clinical Oncology (2013). It has been reported that

IHC has a false negative/false positive rate as high as 20% depending on the quality of the test utilized. FDA approval is not required for IHC- and FISH-based assays. Some tests are developed and performed in Clinical Laboratory Improvement Amendments (CLIA) laboratories.

In their 2013 esophageal and esophagogastric junction cancers guideline, NCCN recommended HER2 testing for "patients with inoperable locally advanced, recurrent, or metastatic adenocarcinoma of the esophagus or esophagogastric junction for whom trastuzumab therapy is being considered." HER2 testing by IHC or FISH is recommended for assessment of overexpression of HER2-neu.

The 2011 Ontario cancer care guidelines on advanced gastric cancer recommendations include the administration of trastuzumab in combination with chemotherapy in patients with advanced gastric cancer who are positive for the HER2/neu receptor (MacKenzie, et al., 2011).

- MPO (myeloperoxidase) is a protein that is considered the hallmark enzyme of the myeloid lineage. The expression of the myeloid gene is specific for myeloid precursors and their leukemic counterparts. A positive stain analysis for MPO is diagnostic of acute myeloid leukemia (also called acute myelogenous leukemia, acute nonlymphocytic leukemia, or ANLL).
- NSE (neuron-specific enolase) is used to help determine the extent of the disease, the patient's prognosis, and the patient's response to treatment in the presence of small cell lung cancer (SCLC). This marker is not used as a screening test for cancer and should not be used alone to distinguish SCLC from non-small cell lung cancers.
- PSA (prostate-specific antigen) is an established marker used in the management of prostate cancer including risk stratification and predicting prognosis. PSA is commonly used as an adjunct to digital rectal exam. (For information on screening refer to the Cigna Coverage Policy Prostate-Specific Antigen (PSA) Screening for Prostate Cancer).
- Thyroglobulin is most often measured in differentiated thyroid cancers (i.e., papillary, follicular, and Hurthle cell). Postoperative elevation of the thyroglobulin level above 10 nanograms per milliliter is suggestive of cancer recurrence.

Bladder Cancer Urine-Based Tumor Markers

Numerous urine-based tumor markers have been proposed for use as an adjunct in the diagnosis and management of bladder cancer. Standard testing includes the non-invasive urine cytology and the gold standard invasive cystoscopy which are typically used for diagnosing and monitoring bladder carcinoma. Following diagnosis and treatment for bladder cancer, urine-based tumor markers may be performed on a routine bases (e.g., every three to six months) to monitor for recurrence. It has been proposed that the use of these bladder markers, especially in combination (e.g., BTA with NMP22 or urine cytology with ImmunoCyte) may enhance specificity and sensitivity producing more reliable outcomes, and are therefore, indicated for the monitoring and/or surveillance of treatment response in patients with bladder cancer. These markers have not been established as a screening tool for bladder cancer.

- Bladder Tumor Associated Antigen (BTA) test, BTA stat® Test and the BTA TRAK® Assay tests are proposed for use in the early detection and monitoring for recurrence of bladder cancer. The BTA stat Test is FDA approved for point-of-care and prescribed in-home use and may be used for the management of bladder cancer patients in conjunction with cystoscopy. These tests are proposed for use in the early detection and monitoring for recurrence of bladder cancer (FDA, 1998).
- ImmunoCyte™/yCyt+™ is an immunocytofluorescence assay FDA approved "for use in conjunction with cytology to increase overall sensitivity for the detection of tumor cells exfoliated in the urine in patients previously diagnosed with bladder cancer". The intent is that the test be used in conjunction with urine cytology and cystoscopy (FDA, 2000).
- Nuclear matrix protein (NMP) 22, NMP22 Test Kit, is FDA approved as an aid in the diagnosis of individuals "with symptoms or risk factors for transitional cell cancer (TCC) of the bladder in conjunction with and not in lieu of current standard diagnostic procedures" and for the management of TCC of the bladder after surgical intervention to identify occult or rapidly recurring disease. The NMP22 BladderChek Test is FDA-approved for point-of-care professionals, as well as prescribed in-home use (FDA, 2002). NMP22 has not gained wide acceptance because of the high rate of false-positive tests and controversy over the optimal cut point for a positive test.

- UroVysion™ Bladder Cancer Kit (UroVysion Kit) is FDA approved for use in conjunction with standard diagnostic procedures “as an aid for initial diagnosis of bladder carcinoma in patients with hematuria and subsequent monitoring for tumor recurrence in patients previously diagnosed with bladder cancer” (FDA, 2005).

Other Bladder Cancer Markers: Several additional urine-based tumor markers are being investigated for use in diagnosing and managing bladder cancer, but their clinical utility has not been established. These include: UBC™ (IDL Biotech, Bromma, Sweden); BLCA-1 and BLCA-2; cytokeratins 8, 18 and 19; fibronectin; hyaluronic acid/ hyaluronidas; Lewis X antigen, microsatellite analysis; quanticyte; soluble fas; survivin (protein and mRNA), and telomerase (e.g., TRAP, hTert, hTR) (Shariat, et al., 2008).

Hyaluronic acid (HA)/hyaluronidase (HAase) testing has been proposed as a diagnostic tool for the screening and detection of bladder cancer. Studies have been primarily in the form of case series with small patient populations using various study protocol, HA assays, and criteria for outcomes (Simpson and Lokeshwar, 2008; Eissa, et al., 2005; Posey, et al., 2003). Lokeshwar et al. (2002) reported 91.0% sensitivity, 70% specificity, 87% accuracy, 92% positive predictive value (PPV), and 67% negative predictive value (NPV) in 70 bladder cancer patients. There were 14 false positives. The evidence in the peer-reviewed scientific literature does not support the accuracy and clinical utility of HA testing, nor have the data shown meaningful improvements in health outcomes.

Kramer et al. (2011) prospectively evaluated the expression of the seven hyaluronic acid (HA) family molecules (i.e., HA-synthases 1, 2, and 3; HYAL-1 hyaluronidase; and HA-receptors [CD44s, CD44v and RHAMM]) from bladder tissues (n=72) and urine specimens (n=148) to compare their diagnostic and prognostic accuracy alone and as a biomarker profile. Quantitative polymerase chain reaction (PCR) and immunohistochemistry analysis were used to measure the expression of the HA molecules. Urine specimens were collected from healthy individuals, and patients with bladder cancer (BCa), benign genitourinary (BGU) conditions or a history of BCa (HXBCa). Mean follow-up was 29.6 ± 5.3 months and median follow-up was 24 months. HYAL-1, CD44v and RHAMM transcript levels were 4–16-fold elevated in BCa tissues compared to normal tissues ($p<0.0001$). CD44 levels were lower. Using univariate and multivariate analyses, tumor stage ($p=0.003$), lymph node invasion ($p=0.033$), HYAL-1 ($p=0.019$) and HAS1 ($p=0.027$) transcript levels and HYAL-1 staining ($p=0.021$) were independently associated with metastasis. Tumor-stage ($p=0.019$) and HYAL-1 ($p=0.046$) transcript levels were associated with disease specific mortality. HA-synthase and HYAL-1 transcript levels were elevated in exfoliated urothelial cells from BCa patients. Combined HAS2-HYAL-1 expression detected BCa with an overall 85.4% sensitivity and 79.5% specificity and predicted BCa recurrence within 6-months ($p=0.004$). HYAL-1 and HAS1 expression predicted BCa metastasis and HYAL-1 expression predicted disease-specific survival. The combined HAS2-HYAL-1 biomarker detected BCa and significantly predicted its recurrence. Author noted limitations of this study included that it was a single institution study with a small number of patients with high-grade BCa with variable clinical follow-up; patients in BCa, BGU and HxBCa categories were not age-matched; and the transcript levels were measured in mixed cell populations. The authors also noted that larger studies with long-term follow-up using an independent set of samples is needed to validate the outcomes of this study.

Professional Societies/Organizations: The National Comprehensive Cancer Network® NCCN® (2013) states that most urine molecular tests for urothelial tumor markers have better sensitivity for detecting cancer that urinary cytology but the specificity is lower and the usefulness of these tests is unclear.

In their discussion of bladder cancer and tumor markers, the ACS stated that NMP22, BTA stat test, ImmunoCyt test and Urovysion may be used to test for cancer but that most physicians felt that cystoscopy is the best way to diagnose bladder cancer. At this time these tests are mainly used to look for bladder cancer in patients who already have signs or symptoms of cancer, or to check for cancer recurrence. According to ACS, 50% of patients with bladder cancer have an abnormal NMP22, but the test seems better suited to check for bladder cancer recurrence. ACS notated that further research is needed before theses tests are used as screening tests (ACS, 2012).

Paraneoplastic Antibodies

While the supporting published evidence is limited, certain paraneoplastic/onconeural antibodies (i.e., anit-Hu, anti-Yo, anti-CV2, anti-RI, anti-MA1 and anti amphiphysin), are established markers used to aid in the diagnosis of paraneoplastic syndromes and occult neoplasms (i.e., cancers of unknown origin). Paraneoplastic

neurological syndromes/disorders (PNS/PND), a rare group of disorders (e.g., limbic encephalitis, progressive cerebellar degeneration), are associated with malignancies but not directly related to the physical effects of the tumor or its metastasis. PNS occurs as a result of damage to the nervous system in the presence of cancer and is thought to arise from an autoimmune response against neuronal antigens (onconeural antibodies) expressed by malignant tumors. It presents in less than 1% of patients with cancer and may be evident prior to the diagnosis of cancer. PNS is most often associated with small cell lung cancer, but can also be present in other cancers (e.g., thymoma and neuroblastoma), and nonmalignant disorders (National Institute of Neurological Disorders and Stroke, 2009; Dalmau and Rosenfeld, 2008; Rugo, 2007; Spiro, et al., 2007; Bataller and Dalmau, 2005; Graus, et al., 2004).

Patients with paraneoplastic antibodies typically present with neurological symptoms (e.g., abnormal balance, coordination, motor skills). If initial diagnostic studies (e.g., laboratory, radiography, cerebral spinal fluid analysis, and/or electromyography) are negative, testing for paraneoplastic antibodies may be warranted. If the test is positive for a paraneoplastic antibody, it may help to focus the search for the neoplasm and establish the diagnosis of cancer. Continued testing (e.g., computed tomography, ultrasound) and early diagnosis for an underlying neoplasm would allow for early treatment of the cancer and could also improve the symptoms of PNS. The diagnosis of PNS is typically made when the neurological syndrome, the associated cancer and the paraneoplastic antibodies are identified. In 90% of patients with paraneoplastic antibodies, the underlying tumor is diagnosed within the first year of PNS symptoms (Dalmau and Rosenfeld, 2008; Spiro et al., 2007; Bataller and Dalmau, 2005).

The specificity of paraneoplastic antibodies reported to be greater than 90% for paraneoplastic neurologic syndromes or some types of cancer makes them useful diagnostic tools. However, not all paraneoplastic antibodies have the same sensitivity and specificity. Hu antibodies, most often associated with subacute sensory neuropathy (SSN) and small cell lung cancer, have an estimated specificity of 99% and a sensitivity of 82% (Dalmau and Rosenfeld, 2008; Honnorat and Antoine, 2007; Vedeler, et al., 2006).

According to an international panel of neurologists, paraneoplastic antibodies are generally categorized as well-characterized or partial characterized. Well-characterized, antibodies are reactive with molecularly defined onconeural antigens, prove the paraneoplastic etiology of the neurological syndrome, and are strongly associated with cancer. The well-characterized paraneoplastic antibodies include: anti-Hu (antineuronal nuclear autoantibodies-1 [ANNA-1]), anti-Yo (PCA-1 [Purkinje cell antibody-1]), anti-CV2 (CRMP5 [collapsing mediator response protein]), anti-Ri (ANNA-2), anti-MA2 (Ta), and anti-amphiphysin. Partially-characterized antibodies are antibodies with an unidentified target antigen and have only been found in a few patients. The partially-characterized antibodies (i.e., antibodies with an unidentified target antigen) include anti-Tr (PCA-Tr), ANNA-3, PCA-2, anti-recoverin, anti-Zic4, anti-mGluR1. The detection of partially-characterized antibodies is considered of limited diagnostic value. Antibodies that can be detected in paraneoplastic and nonparaneoplastic form and can occur with and without cancer include: anti-VGCC (voltage-gated calcium channel), anti-AchR (acetylcholine receptor), anti-nAChR (nicotine acetylcholine receptor), and anti-VGKC (volted-gated potassium channels) (Monstad, et al., 2009; De Graaf and Smitt, 2008; deBeukelaar and Smitt, 2006; Vedeler, et al., 2006; Battler and Dalmau, 2005; Karim, et al., 2005; Vincent, 2005; Graus, et al., 2004).

Professional Societies/Organizations: In their guidelines for small cell lung cancer the National Comprehensive Cancer Network® (NCCN) (2013) noted that “many neurologic and endocrine paraneoplastic syndromes are associated with SCLC [small cell lung cancer]”. In the NCCN 2009 task force report on the management of neuropathy in cancer, NCCN stated that a number of paraneoplastic antibodies have been characterized including anti-CV2/CRMP5 and anit-Hu. NCCN noted that laboratory studies such as the paraneoplastic panel (anti-Hu, anti-Yo, anti Mag) may aid in the diagnosis of PNS.

The National Cancer Institute (2013) stated that patients with SCLC may present with signs and symptoms of paraneoplastic phenomena including inappropriate antidiuretic hormone secretion, paraneoplastic cerebellar degeneration and Lambert-Eaton myasthenic syndrome. NCI (2012) also noted that PNS may be present with thymoma and rarely with thymic cancers.

In their guideline for the initial evaluation of patients with lung cancer, the American College of Chest Physicians (ACCP) (Spiro, et al., 2007) stated that the initial evaluation of the patient should include identification of those

patients with PNS and recommended “that patients with lung cancer and a paraneoplastic syndrome not be precluded from potentially curative therapy on the basis of these symptoms alone”.

Other Tumor Markers
Numerous markers of various types are currently being investigated to determine their accuracy in identifying benign conditions that can lead to cancer, cancer detection, measurement of tumor treatment response, and determination of recurrence. The role of these markers in the management of various benign and cancerous conditions has not yet been established. Research through well-designed, randomized controlled trials is indicated to aid in determining the accuracy and/or clinical utility of additional tumor markers. Many of these tests are developed by a specific laboratory and are not FDA approved.

Autoantibody Panel for Lung Cancer: EarlyCDT™ (Oncimmune, De Soto, KS), Oncimmune™ immune biomarker platform, is a blood test proposed to diagnose the presence of autoantibodies using a panel of seven lung cancer associated antigens in the early stages of the disease. The autoantibodies measured by the test are: CAGE, GBU4-5, HuD, MAGE A4, Ny-ESO-1, p53, and SOX-2. Autoantibodies are stated to be detectable for up to five years before a tumor is visible. The test target population includes individuals age 40–75 years, long term smokers or ex long-term smokers and/or with extensive exposure to environmental factors (e.g., radon, asbestos, radioactive substances) (Oncimmune LLC, 2013). There is insufficient evidence to support the clinical utility of EarlyCDT.

Macdonald et al. (2012) conducted a study to determine if using high throughput (HTP) closing and expression methods would improve the sensitivity and specificity of the EarlyCDT-Lung panel. Serum from two cohorts of lung cancer patients was used (n=269). Sixty-nine proteins were initially screened. Five proteins that displayed cancer and normal differentiation were tested for reproducibility and validated on a separate batch of proteins and patient cohort. Addition of these proteins resulted in an improvement in the sensitivity from 38% to 86% and specificity from 49% to 93%. The high throughput (HTP) method of biomarker discovery improved the performance of the EarlyCDT-Lung panel.

Chapman et al. (2012) conducted a study in an effort to improve the sensitivity of EarlyCDT (n=235). Samples from patients with newly diagnosed lung cancer and matched controls were measured for six tumor-associated autoantibodies (AAbs) (p53, NY-ESO-1, CAGE, GBU4-5, Annexin I, and SOX2) or seven autoantibodies (p53, NY-ESO-1, CAGE, GBU4-5, SOX2, HuD, and MAGE A4). Data were assessed to cancer type and stage. These two panels were compared to two cohorts of patients who were at increased risk for developing lung cancer (n=776 and 836). The six panel test had a sensitivity of 39% and specificity of 89%. The seven panel test had a sensitivity of 41% and specificity of 91% which improved to 93% when adjusted for occult cancers.

Boyle et al. (2011) conducted a clinical validation study for EarlyCDT which included three matched patient cohorts (n=655) with small cell and non-small cell lung cancer. Group one (n=145) included stage I/II lung cancer patients from multiple centers. Group two (n=241) was treated at a single center as part of a collaborative study and group 3 (n=269) was the final validation set and data were run in a blinded manner. Autoantibody levels were measured against a panel of six tumor-related antigens (p53, NY-ESO-1, CAGE, GBU4-5, Annexin 1 and SOX2). The panel yielded a sensitivity/specificity of 36%/91% in group 1, 39%/89% in group two and 37%/90% in groups 3 with good reproducibility. There was no significant difference between lung cancer stages.

CA 50 (Cancer Antigen 50): This antigen has yet to be proven effective in the diagnosis, prognosis, management or surveillance of pancreatic cancer (NACB, 2008).

CA 72-4 (Cancer Antigen 72-4): CA 72-4 is being studied for possible prognostic use and post-operative surveillance of patients who have been diagnosed with ovarian, pancreatic, gastric and colorectal cancer. It detects the presence of AG-72, a mucin-like complex molecule found on the surface of cancer cells. Studies have provided conflicting results and its use is not recommended (ACS, 2012; NACB, 2010; Lee, et al., 2006; Nordenson, 2002).

CA 195 (Cancer Antigen 195): In studies conducted to date, this antigen has yet to be proven effective in the diagnosis, prognosis, management or surveillance of pancreatic cancer (NACB, 2008).

CA 242 (Cancer Antigen 242): Ca 242 has been proposed as a surveillance marker for colorectal cancer. This marker is less sensitive than CEA but may complement CEA. Some preliminary studies have suggested that preoperative concentration of CA 242 may be prognostic of colorectal cancer. However, routine use of CA 242 is not recommended in patients with CRC (NACB, 2009).

CA 549 (Cancer Antigen 549): CA 549 has been proposed as a marker for breast cancer, has been studied in pleural fluids, and used in conjunction with CEA. Its clinical value has not been established (Lee et al., 2006).

CAM 17.1 (Monoclonal Mucin-Based Antibody): CAM 17.1 is an antigen and a mucin-based marker. The diagnostic sensitivity of CAM has not been proven to be as effective as CA 19-9 in studies that have been conducted to date for the treatment of pancreatic cancer (NACB, 2008).

Cathepsin D (Ab-1 Monoclonal Antibody): This is an Ab-1 monoclonal antibody that is currently being studied as a possible marker for use in determining breast cancer prognosis. The results obtained from the studies have been conflicting and this marker is not in clinical use (NACB, 2009; ASCO, 2007).

CYFRA 21-1 (Cytokeratin-19 fragments): This tumor marker has been found in the presence of urological, gastrointestinal, and gynecological cancers. Although some study results are promising for the use of this marker in detecting squamous cell tumors, additional research is needed to determine if its specificity can be useful in the diagnosis and treatment of patients with lung cancer (Lokeshwar, 2005).

DCP (Des-Gamma-Carboxy Prothrombin): DCP, also known as protein induced by vitamin K absence-II (PIVKA-II), is a serum marker under investigation for the detection of hepatobiliary carcinoma. DCP has been proposed as a prognostic factor for recurrence and survival after hepatic resection. Studies are comparing DCP to AFP to determine if DCP is more accurate than AFP or if DCP could be used in conjunction with AFP. The rate of detectable serum DCP is low in patients with small hepatocellular cancers and sensitivity has been reported at 50% (Hakamade, et al., 2008; Wang, et al., 2005). According to the National Comprehensive Cancer Network (2011) and the NACB (2010) DCP is not an established marker for hepatobiliary cancers.

DNA Ploidy (Deoxyribonucleic Acid Ploidy): A DNA ploidy test can measure DNA in tumor cells, and researchers have proposed its use in detecting breast cancer. Studies to date have failed to show its effectiveness in the diagnosis or surveillance of breast cancer. ASCO does not recommend the use of this tumor marker as a prognostic indicator or as a monitor of treatment response in women with breast cancer (ASCO, 2007; Harris, et al., 2007).

DU-PAN-2: Is a sialylated carbohydrate antigen expressed in the presence of pancreatic cancer but its sensitivity and specificity have not been proven to be as good as CA 19.9 (Chung and Podolsky, 2006; Cwik, et al., 2006).

Gene Mutation Marker Panels for Indeterminate Thyroid Nodule Diagnosis
Cytologic examination of fine needle aspirate (FNA) biopsy is considered the “gold standard” for preoperative diagnosis of a thyroid nodule. Approximately 15%–30% of thyroid biopsies are indeterminate and 15–30% of indeterminant nodules are malignant. The treatment for indeterminant nodules typically involves surgical intervention. In order to avoid surgery in patients with a benign indeterminant FNA, evaluation of FNA biopsies using individual genetic markers or a panel of markers are being investigated to aid in the diagnosis of cancer. It has been established that BRAF and RAS mutations and RET/PTC and PAX8/PPARy rearrangements account for the majority of molecular alterations detected in differentiated thyroid cancers. Although the detection of these molecular alterations appears to have diagnostic utility especially in indeterminate nodules, there are insufficient sensitivities, particularly for follicular cancers. The percentage of false negative and false positive results remains a challenge. The panels are used as an adjunctive test to clinical evaluation and other diagnostic testing (Albarel, et al., 2012; Ferraz et al., 2011; Wang, et al, 2011). There is insufficient evidence to support the accuracy and clinical utility of these tests.

miRInform® Thyroid (Asuragen®, Austin, TX) is a panel of molecular DNA and RNA based markers proposed to improve preoperative diagnosis of indeterminate nodules and aid in the characteristics of malignancy. The test includes 17 markers including seven KRAS, one BRAF, three HRAS and three NRAS mutations and three RNA fusion transcripts. The presence of these markers is known to be correlated with malignant thyroid nodules.

According to the manufacturer the sensitivity of cytology alone is 44–66% compared to 80–90% sensitivity with the addition of miRInform. The analytical sensitivity is 95% and the specificity is 99%. Asuragen notes that the clinical sensitivity and specificity for this test have not been established. A positive result indicates at least one of the markers was detected and a negative result indicates that none of the markers was detected. However, a negative result does not mean the nodule is benign. Asuragen is a CLIA certified and College of American Pathology (CAP) accredited laboratory (Asuragen, 2013).

Quest Diagnostics® offers a Thyroid Cancer Mutation Panel which identifies mutations of BRAF V600E, RAS, RET/PTC and PAX8/PPAR gamma molecular markers. These markers are known to be associated with papillary and follicular thyroid cancer. The test may be used on indeterminate FNA biopsies to aid in the diagnosis of cancer. A study conducted by Quest found that 90 of 149 FNA specimens (60%) had mutation of one or more of the four markers (Quest, 2012).

Ferraz et al. (2011) evaluated 20 studies that used molecular analysis of fine needle aspirate biopsy (FNAB) for the diagnosis of thyroid malignancy. Sixteen studies analyzed one mutation including BRAF and RET/PTC and four studies analyzed several mutations (e.g., BRAF, RAS, RET/PTC and/or PAX8/PPARg). The authors calculated the false-positive, false negative, sensitivity, and specificity rates of the studies that used indeterminate/follicular lesions, follicular lesions of indeterminate significance/atypia of indeterminate significance FNAB category samples by comparing the result of the FNAB mutation detection with the final histology. The detection of a mutation in a histologically benign thyroid lesion was categorized as a false positive. Detecting no mutations in an FNAB sample from a histologically benign surgical sample was considered a true negative and no mutation in a histologically malignant lesion was considered a false negative. Means from studies classified according to type and number of mutations and FNAB category for indeterminate samples were: 1.25% (range, 0%–4%) false positive, 9% (range 1%–21%) false negative, 38%–85.7% sensitivity and 95–100% specificity. Limitations of the sampling included using part of the same sample for cytology and molecular analysis (e.g., leftover cells in needle, needle washing, or part of the total FNAB) or not using the same sample for both analyses. Some studies used fresh FNAB samples and some used routine air-dried fine-needle aspiration. Cytological classification, methodology, number of samples, method of extraction and conservation of material varied from study to study. Some studies combined suspicious and indeterminate samples in the same category and some did not. The authors noted that “the current level of molecular analysis in FNAB is still restricted to a few specialized laboratories. There is a need for certified laboratories, adequate material, sensitive and standardized methods for extracting DNA and especially mRNA and microRNA (miRNA) from routine air-dried smear samples, and also a need for sensitive methods for mutation detection before it is possible to introduce molecular FNAB cytology diagnostics in the daily routine thyroid nodule workup”.

Nikiforov et al. (2011) conducted a molecular analysis on 1056 previously collected consecutive FNA samples to investigate the clinical utility of molecular testing for thyroid cancer on indeterminate cytology. A PCR assay and testing for a panel of mutations consisted of BRAF V600E, NRAS codon 61, HRAS codon 61, and KRAS codons 12/13 point mutations and RET/PTC1, RET/PTC3, and PAX8/PPARγ rearrangements. A total of 967 adequate samples (92%) yielded 87 mutations including 19 BRAF, 62 RAS, one RET/PTC, and five PAX8/PPARγ, and 479 patients underwent surgery. In nodules with indeterminate cytology including atypia of undetermined significance/follicular lesion of undetermined significance, follicular neoplasm/suspicious for a follicular neoplasm, and suspicious for malignant cells, the detection of any mutation conferred the risk of histologic malignancy of 88, 87, and 95%, respectively. The risk of malignancy based on cytology only was 14%, 27% and 54% respectively. The risk of cancer in mutation-negative nodules was 6, 14, and 28%, respectively. Of the 6% of cancers in mutation-negative nodules with atypia of undetermined significance/follicular lesion of undetermined significance cytology, 2.3% were invasive and 0.5% had extrathyroidal extension. Based on the various types of cancer (n=513), the accuracy was 81%-94%, sensitivity 57%–68%, specificity 96%–99%, positive predictive value was 87–95%, negative predictive value 72%–94% for predicting thyroid cancer. The BRAF V600E, RET/PTC, and PAX8/PPARγ mutations were associated with malignancy in close to 100% of nodules. The results of this study indicated that mutational testing using residual material obtained during routine FNA allows more accurate cancer risk stratification of cytologically indeterminate nodules which may be used to alter the surgical intervention for the patient.

Ohori et al. (2010) retrospectively analyzed FNA samples of follicular lesion of undetermined significance (FLUS)/atypia of undetermined significance (AUS) (n=117) to compare cytologic findings with molecular studies and pathology results. Analysis for BRAF and RAS gene mutations and RET/PTC and PAX8/PPARγ gene

rearrangements were performed and correlated with the cytologic-histologic outcome. Twelve positive molecular cases with papillary cancer were found. When the molecular results were compared to surgical pathology, the cancer probability for FLUS/AUS cases with molecular alteration was 100%, compared to 7.6% ($p<0.001$) probability without molecular alteration. There were no false positives.

The 2013 National Comprehensive Cancer Network® (NCCN) guidelines on thyroid cancer, and the NCCN Biomarker Compendium™ (2012) rate molecular testing for a subset of thyroid cancers as a category 2A (based upon lower-level evidence, there is uniform consensus that the intervention is appropriate). According to NCCN molecular testing to detect individual mutations (e.g., BRAF, RET/PTC, RAS, PAX8/PPAR [peroxisome proliferatory-activated receptors] gamma) or pattern recognition approaches using molecular classifiers may be useful in the evaluation of indeterminate FNA samples. The NCCN recommendations include molecular diagnostics for evaluating 1) FNA results that are suspicious for Follicular or Hurthle cell neoplasms or 2) follicular lesion of undetermined significance. Results of molecular testing may allow for observation of the tumor as opposed to immediate surgical resection if the application of the test results in a predicted risk of malignancy that is comparable to the rate seen in cytologically benign thyroid FNAs (approximately $\leq 5\%$). Due to the lack of clinical trials involving pediatric patients, this recommendation only applies to adults.

The American Thyroid Association (ATA) Clinical Affairs Committee (Hodak and Rosenthal, 2013) published an official statement to provide direction for clinicians and patients regarding the current state of thyroid molecular diagnosis including Afirma, miRInform and Cleveland Clinic TSHR mRNA Assay. ATA stated that the commercial and noncommercial use of BRAF, RAS, RET/PTC, and PAX8/PPARγ testing have promising roles, but experience with these tests is limited and"no test has perfect sensitivity and specificity". ATA stated that until expert consensus review of existing data is completed, no evidence-based recommendation for or against the use of these tests can be made. They advised clinicians to use caution and to remain cognizant of the limited available data. "Until evidence-based recommendations are available, determining whether or not the limited data available support the use of these methods should be considered on a case-by-case basis".

In their guidelines for the management of thyroid nodules, the American Association of Clinical Endocrinologist (AACE) (2010) stated that currently, no single cytochemical or genetic marker is specific and sensitive enough to replace the morphologic diagnosis of follicular lesion or suspicious for neoplasm. AACE noted that it is possible to identify specific gene alterations (e.g., p53, Ras, erb2, p27) of FNA biopsy specimens but they are not used in daily cytologic practice. Since no specific marker is 100% sensitive and 100% specific, a panel of markers (e.g., HBME-1, cytokeratin 19, and galectin-3) may provide diagnostic accuracy in cytologic diagnosis. AAEC did not discuss the use of gene expression classifiers.

In a summary statement on the utility of molecular marker testing in thyroid cancer, the American Association for Endocrine Surgeons (Yip, et al., 2010) stated that the use of molecular markers into clinical algorithms is still evolving and studies are needed to identify how routine molecular testing can best complement cytology and ultrasound and better understand the prognostic significance of a positive test.

GCC (Guanylyl Cyclase C): GCC or GUCY2C is a heat-stable enterotoxin receptor that may be useful as a marker to identify patients with colorectal cancer or early pre-malignant changes in the upper gastrointestinal tract. GCC is normally expressed in the intestinal tract on the luminal side of the intestinal epithelial cells, and it persists after neoplastic changes occur (Schulz, et al., 2006). An example of a GCC test is the Previstage™ GCC Colorectal Cancer Staging Test (DiagnoCure Oncology Laboratories, Quebec QC, CAN).

HER2 Gene Amplification Testing of Breast Cancer Tissue (e.g., SPoT-Light® HER2 CISH™): Spot-Light HER2 CISH (Invitrogen Corp, Carlsbad, CA) received FDA premarket approval to "quantitatively determine HER2 gene amplification in formalin-fixed, paraffin-embedded (FFPE) breast carcinoma tissue sections using Chromogenic in Situ Hybridization (CISH) and brightfield microscopy". The test "is indicated as an aid in the assessment of patients for whom Herceptin® (trastuzumab) treatment is being considered. The assay results are intended for use as an adjunct to the clinicopathological information currently being used as part of the management of breast cancer patients. Interpretation of test results must be made within the context of the patient's clinical history by a qualified pathologist". The test is proposed to detect hybridization of labeled nucleic acid probes using conventional peroxidase-diaminobenzidine (DAB) reactions, allowing the pathologist to see tissue morphology and gene aberrations simultaneously (FDA, 2008). CISH is an evolving technology that is also purposed to incorporate advantages of both IHC and FISH. There is insufficient evidence in the published

peer-reviewed scientific literature to support the accuracy, clinical utility, and impact on meaningful health outcomes of Spot-Light HER2 CISH.

Madrid and Lo (2004) conducted a retrospective review to assess the accuracy of the Spot-Light CISH assay (n=160) compared to results of previously reported IHC analysis in tumor specimens from women with breast cancer. The specimens were divided into the four IHC scores, 0–1+ (negative), 2+ (equivocal), and 3+ (positive) (n=40 samples per group), and compared to the CISH low and high risk scores. The concordance between CISH and IHC 0–1+ and 3+ tumors was 100% each, 45% on IHC 2+ tumors, 72.50% on all positive IHC tumors, and overall concordance was 86.25%. All IHC negatives were CISH non-amplified and all IHC 3+ were CISH amplified. The authors acknowledged that the clinical utility of the low- and high-amplified CISH results has not been established.

hMAM or MG (Human Mammoglobulin): hMAM is a gene which encodes similar to epithelial secretory proteins which are part of the uteroglobin family. Its expression has been linked to epithelial breast tumor cells. There is minimal information in the literature that demonstrates the clinical utility of hMAM expression or patient net health outcomes from its clinical application (Nunez-Villar, 2003).

LASA-P (Lipid Associated Sialic Acid in Plasma): LASA-P has been studied as a possible marker for epithelial ovarian cancer as well as several other cancers. This marker has not been proven to be valuable in the detection of cancer. LASA-P is not specific for any particular cancer or even for cancer in general, as it can also be elevated in some noncancerous conditions. It may be occasionally used along with other markers to follow response to treatment, but CA 125 is the standard marker (ACS, 2012).

LPA (Lysophosphatidic Acid): LPA is a “multifunctional signaling molecule in fibroblasts and other cells” and has been found in patients with ascitic fluid due to ovarian cancer cell proliferation (Hussain, 2012). In their discussion of genetics of breast and ovarian cancer, NCI stated that studies have included small patient populations and “comprise highly-selected known ovarian cancer cases and healthy controls of the type evaluated in early biomarker development phases I and II. Results have not been consistently replicated in multiple studies; presently, none are considered ready for widespread clinical application” (NCI, 2012).

MCA (mucin-like carcinoma antigen): MCA is a glycoprotein oncofetal antigen related to tumor burden, milk production and fetal development that has been proposed as a tumor marker for breast cancer. The low specificity of MCA limits its clinical application (Sarandakou, et al., 2007; Nicolini, et al., 2006).

MCAM (Melanoma Cell Adhesion Molecule): This molecule is an integral membrane glycoprotein with Ca^{2+} independent cell adhesive properties, which can result in dynamic actin-cytoskeleton rearrangements. This activity can lead to cell detachment and migration which are key functions in metastases and invasion of cancers. The presence of this glycoprotein has been noted in the presence of several cancers, and promising findings have been noted in its relation to melanoma. Its use is currently under investigation to determine its ability to be utilized as a melanoma tumor marker (NACB, 2008).

Methylation Analysis of DNA for Determining Tumor Grade: DecisionDX-G-CIMP (Castle Biosciences Inc., Phoenix AZ) is a multi-methylation assay that is proposed to determine the glioma-CpG island methylator (G-CIMP) of grade 2, 3, or 4 gliomas which would aid the physician in determining the treatment options for a patient. The glioma-specific CIMP profile was developed using 272 glioma samples from a cancer genome project and validated on more than 486 glioma samples. DecisionDX-LGG is reported to be an independent predictor of survival ($p<0.0003$). The test is performed by Castle Biosciences, a CLIA-certified laboratory (Castle Biosciences, 2011). There is insufficient evidence in the published peer-reviewed literature to support the clinically utility of this methylation assay.

Microarray Analysis for Measuring the Degree Of Similarity in Undifferentiated Tumor Types (e.g., Pathwork® Tissue Of Origin Kit: The Pathwork® Tissue of Origin (TOO) (Pathwork Diagnostics, Inc., Redwood City, CA) is a 510(k) FDA-approved microarray-based gene expression assay intended to “measure the degree of similarity between the RNA expression patterns in a patient's formalin-fixed, paraffin-embedded (FFPE) tumor and the RNA expression patterns in a database of fifteen tumor types (poorly differentiated, undifferentiated and metastatic cases) that were diagnosed according to then current clinical and pathological practice. This test should be evaluated by a qualified physician in the context of the patient's clinical history and other diagnostic

test results". The test is "not intended to establish the origin of tumors (e.g. cancer of unknown primary) that cannot be diagnosed according to current clinical and pathological practice" nor is it intended to "subclassify or modify the classification of tumors that can be diagnosed by current clinical and pathological practice, nor to predict disease course or survival or treatment efficacy, nor to distinguish primary from metastatic tumor". Pathwork uses the Pathchip technology (Affymetrix, Inc., Santa Clara, CA) which is a custom-designed microarray that uses oligonucleotide features to analyze the tissue samples (FDA, 2010). There is also an FDA approved Pathwork Tissue of Origin Test for frozen biopsy specimens (FDA, 2008).

The assay measures the expression of 1550 genes in each sample. An algorithm using a list of markers, a set of reference genes, and a set of coefficients were combined to produce 15 Similarity Scores (ranging from 0–100), one for each of the possible tissues on the test panel. The algorithms used and the processing methods for FFPE are not the same as for frozen specimens. The higher the score, the more likely the tissue corresponds to the molecular marker (i.e., reference tissue). The 15 tumor types that the study analyzes include: bladder, breast, colorectal, gastric, hepatocellular, kidney, melanoma, non-Hodgkins lymphoma, non-small cell lung, ovarian, pancreatic, prostate, sarcoma, testicular germ cell tumor, and thyroid (FDA, 2010; FDA, 2008). The proposed utility of the test is that the ability to identify the tissue of origin would increase the chances of the patient receiving more targeted, less toxic therapy; enhance optimal management; and reduce the amount of time and expense diagnosing the primary tumor (Pillai, et al., 2011; Monzon, et al., 2010).

To verify the analytical and clinical performance of the Pathwork Tumor of Origin (TOO) Test, Dumur et al. (2011) conducted a retrospective review of tissue samples from selected cases (n=40) that were considered difficult to diagnose. Six specimens with diagnoses not covered by the test panel of 15 tissue types ("off-panel"cases) and seven specimens diagnosed as cancers of unknown primary (CUP) were intentionally included. The remaining 30 specimens corresponded to 20 metastases and 10 primary tumors, with at least one of the possible diagnoses corresponding to one tissue type covered by the test panel. Discordant results between the Pathwork and the original diagnosis were further evaluated by immunohistochemical analysis. Three cases not meeting specifications were excluded. Twenty-nine samples were diagnosed with at least one TOO. The 29 tumors included 10 different tissue types of the possible 15 types identified by Pathwork. There was agreement in 79% of TOO cases compared to immunohistochemical analysis. Of the six discordant cases, results from subsequent clinical and immunohistochemical analysis yielded results that concurred with Pathwork results. Of the six off-panel cases, Pathwork defined two as indeterminate and the other four results did not match the clinical and histopathic findings. A 97% agreement was reported when account of subsequent clinical findings and immunohistochemical findings for six disconcordant cases was reviewed. Limitations of the study include the small patient population and retrospective study design.

Pillai et al. (2011) conducted a multicenter validation study and an interlaboratory reproducibility study for Pathwork Tissue of Origin Test using metastatic, poorly differentiated or undifferentiated FFPE tumor specimens (n=462). The reference diagnoses were masked. An algorithm developed by the authors, quantified the similarity between RNA expression of the study specimens and the 15 tissues on the test panel. Overall agreement with the reference diagnosis was 88.5%, the positive percent agreement was also 88.5% and the negative percent agreement was 99.1%. The study reported the following concordance between the reference diagnosis and Pathwork-FFPE diagnosis: bladder 79.3% (n=29); breast 96.5% (n=57); colorectal 91.7% (n=36); gastric 72.0% (n=25); hepatocellular 96.0% (n=25); kidney 89.3% (n=28); melanoma 84.0% (n=25); non-Hodgkin's lymphoma 89.7% (n=29); non-small cell lung 85.2% (n=27); ovarian 88.9% (n=45); pancreas 85.7% (n=28); prostate 96.0% (n=25); sarcoma 88.9% (n=27); testicular germ cell 84.0% (n=25); and thyroid 90.3% (n=31). Agreement with metastatic specimens (n=179) was 91.1% and 86.9% for undifferentiated primary tumors (n=283). The reproducibility concordance of the test results (n=149 paired results) performed in three laboratories was 89.3%. Management of the patients based on the results of the test was not discussed.

For FFPE testing, overall accuracy (n=162 total samples) tested by three laboratories was 82.1%. Agreement/non-agreement was reported by tissue type and agreement ranged from 79.3% (bladder) to 96.5% (breast). Overall agreement to available diagnosis was 88.5% and non-agreement was 11.5%. Agreement for metastatic tumors was 91.1%, and non-agreement was 8.9%. Agreement for poorly and undifferentiated primary tumors was 86.9% and non-agreement was 11.5% (FDA, 2010). There is insufficient evidence in the published peer-reviewed literature to support the clinical utility of Pathwork and its impact on meaningful health outcomes.

Using the Pathwork Tissue of Origin test, Monzon, et al (2010) retrospectively measured gene expression of fresh-frozen specimens from 21 patients with carcinoma of unknown primary (CUP). The specimens were obtained from tissue bank archives and taken from over a dozen biopsy sites. The study aims were to "evaluate the test's ability to issue a clear positive call in classic CUP specimens, to check the consistency of the test results against a short-list of diagnostic possibilities based on clinicopathology, and to estimate the potential added clinical value of positive and negative results in guiding management". Pathwork reported a positive single tissue in 16 (76%) specimens and identified 16 primary sites (i.e., five colorectal, four breast, three ovary, two lung and two pancreas). Five cases were indeterminate (24%). The Pathwork results were consistent with 10 (62%) clinicopathologic suggestions. On average, the test ruled out 11 tissue types per case. The small patient population and retrospective design preclude the ability to draw any conclusions from this study.

Monzon et al. (2009) conducted a blinded multicenter validation study (n=547) of the Pathwork test using a 1550 gene expression profile. The study included 351 frozen specimens obtained from a tissue bank and 271 electronic files of microarray data from the International Genomics Consortium. The specimens were histologically verified and included metastatic tumors and poorly differentiated or undifferentiated primary tumors. Overall agreement was 87.8%, positive percent agreement (sensitivity) was 87.8% and the negative percent agreement (specificity) was 99.4%. Overall rate of non-agreement was 7.1% and the rate of indeterminate calls was 5.1% (n=28). Highest rate of agreement was for breast cancer (94.1%) (n=68) and the lowest was 72% for gastric and pancreatic cancers (n=25 each). Performance for the test was significantly better for primary tumors (90.7% agreement) (n=289) than for metastatic tumors (84.5% agreement) (n=258) (p=0.04). There were no significant differences in the rates of agreement between the three laboratories. Author noted limitations of the study included: the "inability to independently verify the reference diagnosis used to assess the accuracy of the test" (i.e., diagnoses were taken from the surgical pathology report at the time the tissue was banked); due to blinding, the pathologist was unable to consult with the treating physician, and the test is designed to be interpreted with clinical information; and there is a possibility that an uncertain primary cancer could originate from a site that is not included in the Pathwork test.

In their clinical practice guidelines on occult primary cancer (i.e., CUP), NCCN (2012) stated that "gene signature profiling for tissue of origin is not recommended for standard management at this time". The data is insufficient to determine if testing would improve prognosis.

MicroRNA Testing: MicroRNA testing has been proposed for aiding in the differentiation or diagnosis of various types of cancers. Studies supporting the accuracy, clinical utility and impact on meaningful health outcomes of microRNA testing for these indications are lacking.

ProOnc SquamousDx™ (Prometheus Laboratories, Inc., San Diego CA) is proposed to differentiate squamous from non-squamous non-small cell lung cancer (NSCLC) based on miRNA hsa-miR-205 expression levels which may be over expressed in squamous cancers. The hypothesis is that once the cell type is identified, treatment can be targeted to the specific cancer type (Prometheus, 2010). In a validation study, using quantitative real-time polymerase chain reaction (qRT-PCR), Lebanony et al. (2009) reported that the sensitivity of identifying squamous cell carcinoma with has-miR-205 expression was 96% (23 of 24 samples) with 79% classified as squamous with high confidence. The specificity of the test for classifying samples as nonsquamous was 90% (44 of 49 samples).

ProOnc Mesothelioma$^{Dx™}$ (Prometheus Laboratories, Inc., San Diego CA) is proposed to aid in the diagnosis of mesothelioma based on the expression level of three microRNAs, hsa-miR-193-3p, hsa-miR-200c, hsa-miR-192. The test is purported to differentiate mesothelioma from other lung and pleura carcinomas (Prometheus, 2010).

ProOnc TumorSource$^{Dx™}$ (Prometheus Laboratories, Inc., San Diego CA) is described as using 48 miRNAs to identify the tissue of origin of a metastatic tumor, also called unknown primary cancer, occult primary malignancy, or occult primary tumor, from fresh-frozen or formalin-fixed paraffin-embedded (FFPE) tissue. The test is proposed to identify 25 classes of tissue origin including breast, colon, lung, prostate, ovarian and kidney. Using a dual algorithm approach of decision tree and K Nearest Neighbor analyses, concordant results were reported as having occurred with 66% of samples with 80%–84% overall sensitivity of tissue-of origin/histological type and 87%-90% sensitivity of single predicted origin (Prometheus, 2010).

Multigene Expression Testing: Multigene expression testing has been proposed as a platform for genetic profiling for predicting colon cancer recurrence, for determining the molecular signature of a glioblastoma multiform (GBM) tumor and for classifying a uveal melanoma tumor as low or high risk.

The 12-gene Oncotype DX® Colon Cancer Assay (Genomic Health, Redwood City, CA) reverse transcriptase polymerase chain reaction (RT-PCR) assay is a proposed method of predicting the risk of stage II colon cancer recurrence and aiding in the decision regarding adjuvant chemotherapy. This is done through the reporting of an individualized Recurrent Score (RS) that ranges from 0-100. The RS is based on the quantitative expression of seven cancer genes (i.e., Ki-67, MYBL2, C-MYC, FAP, INHBA, BGN and GADD45B) and five reference genes (i.e., ATP5E, PGK1, GPX1, UBB, VDAC2). The test is only applicable to newly diagnosed adenocarcinoma or mucinous stage II colon cancer patients who have undergone surgical resection. It has been suggested that patients with a high RS (i.e. $\geq$ 41) are the best candidates for adjuvant therapy. The assay is performed on paraffin-embedded primary colon tumor tissue by Genomic® Health's clinical laboratory improvement amendments (CLIA)-certified, College of American Pathologists (CAP)-certified laboratory (Genomic Health, 2013; Webber, et al., 2010). Studies supporting the accuracy, clinical utility and impact on meaningful health outcomes of Oncotype DX Colon Cancer Assay are lacking (Marshall, 2010; Midgley, et al., 2010; Rasul and Kerr, 2010; Webber, et al., 2010). The role of this testing in patient management has not been established.

Yothers et al. 2013 conducted an independent prospectively designed study using archived specimens (n=892) from the National Surgical Adjuvant Breast and Bowel Project (NSABP) C-07 clinical trial to clinically validate Oncotype DX continuous Recurrence Score (RS). Specimens were taken from resected stage II and stage III colon cancer patients who were randomly assigned to receive fluorouracil (FU) or FU plus oxaliplatin. The primary end point was recurrence-free interval (RFI) (i.e. time from random assignment to first colon cancer recurrence). Recurrence at death found on autopsy was considered a recurrence event. Of the 892 patients, 31/264 stage II and 214/628 stage III patients had recurrence. The continuous RS was significantly associated with RFI and significantly predicted recurrence ($p<0.001$), disease-free ($p<0.001$) and overall survival ($p<0.001$). After adjustment for stage, mismatch repair, nodes examined, grade and treatment, the RS predicted recurrence risk ($p=0.001$). There were no significant interactions between the RS and stage of cancer or age of the patient. The relative benefit of oxaliplatin was similar across all RS ranges ($p=0.48$). The higher the RS, the higher the absolute benefit from oxaliplatin, especially in patients with stage II and stage IIIA/B tumors. According to the authors, the RS does not predict oxaliplatin treatment efficacy and cannot be used to identify patients for whom oxaliplatin has no benefit. When the RS is combined with stage, it provides better discrimination of recurrence risk and may enable better discrimination of absolute benefit as a function of risk. Patient with a higher recurrence score would be expected to derive a larger absolute treatment benefit than low risk patients. The results of this study do not allow RS for patient with mismatch repair gene (MMR) deficient stage III tumors.

Venook et al. (2013) conducted a validation study (n=162) of the 12-gene Recurrence Score (RS) using patient specimens from the Cancer and Leukemia Group B (CALGB) 9581 randomized controlled trial. The CALBG study found no effect of adjuvant edrecolomab compared with observation in patients with resected stage II colon cancer. Out of 1672 patients in the CALGB 9581 study, 690 specimens were available for evaluation of which 162 had recurrence. A random 1:3 selection of nonrecurring patients was included. The primary end point was recurrence-free interval (RFI) (i.e. time from random assignment to first colon cancer recurrence) or death from colon cancer. Continuous RS was significantly associated with the risk of recurrence ($p=0.013$) and mismatch repair (MMR) gene deficiency ($p=0.044$). RS was significantly predictive ($p=0.004$) and the strongest predictor of recurrence compared to tumor stage and grade, MMR, nodes examined, and lymphovascular invasion. The most common subset was patients with T3 MMR-I tumors and the RS appeared to be independently associated with recurrence in this subset.

Gray et al. (2011) conducted a validation study (n=1436) to determine if a multigene RT-PCR clinical assay (i.e., Oncotype DX Colon Cancer Assay) could provide clinical information (i.e., recurrence and treatment benefit) to assist in making treatment decisions for patients with resected stage II colon cancer. Expression levels of 761 candidate genes from four studies involving patients with resected stage II and stage III colon cancer were measured. Analysis of pooled data from surgery alone (n=2 studies) and from surgery plus adjuvant fluorouracil (FU)/folinic acid (FA) chemotherapy (n=2 studies) studies was performed. The final gene list was then selected. Algorithms based on seven genes for recurrence score and six genes predictive of FU/FA benefit for treatment scores were devised. Five reference genes were also included. Using a prospectively defined treatment score, validation of the multigene RT-PCR assay was attempted using RNA from 1436 fixed paraffin-embedded tumor

blocks from the QUASAR (Quick and Simple Reliable) study. The QUASAR study evaluated the outcomes of surgery alone compared to surgery plus adjuvant fluoropyrimidine chemotherapy. The risk of recurrence was significantly associated with the RS per interquartile range (p=0.04). The recurrence risk at three years was 12% for low risk (RS< 30), 18% for intermediate risk (RS 30–40) and 22% for high risk (RS≥ 41). The strongest histopathologic prognostic factors were tumor stage and mismatch repair (MMR) (p<0.001, each). The continuous RS was significantly associated with risk of recurrence beyond the histopathologic prognostic factors (p=0.006). The TS showed no increased benefit from chemotherapy at a higher score (p=0.95). The outcomes suggested that the RS in combination with the tumor stage and MMR would provide additional prognostic value. Independent studies to support the outcomes of this study and to establish clinical utility of the test are indicated.

Clark-Langone et al. (2010) reported the design and analytical validation results of the Oncotype DX Colon Cancer Assay. The 12-gene assay was selected from 761 genes based on analysis of fixed paraffin-embedded (FPE) colon cancer (Stage II adenocarcinoma and mucinous carcinoma) tissue samples (n> 1800) from four development studies. Validation outcomes included the following: amplification efficiencies ranged from 96%–107%. Results greater than 100% may have been caused by pipette error, the presence of PCR inhibitors or non-specific amplification; the linearity, analytical sensitivity and analytical precision met acceptance criteria; all the genes in the panel met the pre-specified acceptance criteria for reproducibility; finally a control sample was run on 21 reverse transcription plates, seven PCR plates failed (< 2%).

In their colon cancer guidelines, NCCN (2013) states that there is “insufficient evidence to recommend the use of multi-gene assay panels to determine adjuvant therapy” and questions the value that they add.

The DecisionDx-GBM (Castle Biosciences Inc., Phoenix AZ) is a multigene expression assay proposed to allow stratification of a tumor’s response to first-line therapy (i.e., radiation and temozolomide) in a newly-diagnosed patient with a grade IV GBM and predict which patients are likely to experience greater than two-years of progression free survival. The assay uses reverse transcriptase polymerase chain reaction (RT-PCR) to determine the expression of a 12-gene panel (i.e., AQP1, CHI3L1, EMP3, GPNMB, IGFBP2, LGALS3, OLIG2, PDPN, RTN1 and three control genes, EEF1A1, GUSB, RPS27). Validation studies (n=169) reported that DecisionDX-GBM was significantly predictive of progression-free (p=0.0003) and overall survival (p=0.003). It is proposed that a patient with a DecisionDX score within the first quintile has a likelihood of survival for up to two years. The hypothesis is that if the patient is not predicted to survive for two or more years the treatment plan would be altered and not include first-line therapy. The test is performed by Castle, a CLIA-certified laboratory (Castle Biosciences Inc., 2013; Allington-Hawkins, et al., 2010; Colman et al., 2010). There is insufficient evidence in the published peer-reviewed literature to support the accuracy and clinical utility of DecisionDx, and its impact on meaningful health outcomes.

Allingham-Hawkins (2010) conducted a systematic review to evaluate the accuracy and clinical utility of the DecisionDx-GBM test. Only one study directly related to this test was found. The authors reported the following: no information regarding analytical validity of the test was identified; test accuracy and reliability had been published in one study (n=110) and validation with a larger set is needed; the proprietary algorithm used to generate the risk score for the test had not been independently validated; and no data were found regarding net benefit of the test in improving heath outcomes. In conclusion, there is insufficient evidence to recommend this test for routine use in this patient population.

Colman et al. (2010) used four independent data sets to identify a multigene set predictive of survival in GBM patients. Microarray analysis of formalin fixed-paraffin embedded (FFPE) tumor (n=279) resulted in a nine-gene subset with the highest survival associations. The gene subset was then validated in patients (n=101) treated with radiation and concurrent and adjuvant temozolomide and was also tested for MGMT methylation. There was a significant correlation in progression-free survival (PFS) for the gene profile (p=0.0007) and the overall survival (p=0.0055). Determination of MGMT promoter methylation status (a predictor of outcome in patients treated with this regimen) showed an association with PFS, but was not shown to be an independent survival predictor in this study.

Castle Biosciences also offers a Decision DX-UM (uveal melanoma) 15-gene assay that is proposed to analyze the molecular structure of a UM to differentiate between a low-risk tumor (i.e., class 1 molecular signature) and a high-risk tumor (i.e. class 2 molecular signature). Class 2 tumors are proposed to be most likely associated

with metastasis outside of the globe (e.g., to the liver). The specimen can be obtained from a needle biopsy performed prior to treatment or from a formalin-fixed, paraffin-embedded specimen (FFPE). The original microarray platform was later migrated to a polymerase chain reaction (PCR)-based platform TaqMan (Castle Biosciences, 2013; Onken, et al., 2010).

Onken et al. (2012) prospectively obtained 459 posterior uveal melanoma tumor samples from 15 centers to evaluate the prognostic performance of a 15-gene expression profiling (GEP) assay including 12 discrimination genes and 3 control genes. A Collaborative Ocular Oncology Group (COOG) from 12 ocular oncology centers was formed to conduct this study (COOG Study). Tumor samples were obtained by FNA (n=359), post-enucleation FNA (n=92) and local tumor resection (n=8). Median follow-up was 17.4 months (mean, 18.0 months). The GEP assay classified 459 cases (97.2%). A total of 276 (61.9%) were class 1 and 170 (38.1%) were class 2. Metastases were detected in significantly more class 2 patients than class 1 patients (44 vs. 3) ($p<0.0001$). GEP was the only prognostic factor including age, ciliary body involvement, tumor diameter, tumor thickness, tumor cell type, and chromosome 3 status associated with metastasis that contributed independent prognostic information ($p=0.006$). A significant association was reported with tumor, node, and metastasis (TNM) status ($p=0.003$). Because monosomy 3 has been used as a prognostic marker for uveal melanoma, GEP was compared to chromosome 3. GEP was more strongly associated with metastasis than chromosome 3 status among the discordant cases ($p<0.0001$). Because of the inferior prognostic value of chromosome 3 status, chromosome 3 testing was discontinued after the first 260 cases. Chromosome 3 status did not contribute additional prognostic information that was independent of GEP ($p=0.2$).

Worley et al. (2007) conducted a validation study to compare the prognostic accuracy of a gene expression–based classifier to monosomy 3, a standard genetic prognostic marker, detected by FISH and CGH (n=67). Patients had undergone enucleation for various UM tumor types and 18 had experienced metastases. Gene profiling was performed on 52 tumors and assigned 27 tumors as class 1 and 25 tumors as class 2. Statistically, class 2 signatures ($p=0.0001$), advanced patient age ($p=0.01$), and scleral invasion ($p=0.007$) were significantly associated with metastasis. The sensitivity for the molecular classifier was 84.6% and the specificity was 92.9% compared to 58.3% and 85.7% for monosomy 3 microarray analysis, respectively and 50.0% and 72.7% for monosomy 3 FISH analysis, respectively. The positive predictive value (PPV) to the classifier was 91.7% and the negative predictive value (NPV) was 86.7%. Per the authors the PPV and NPV were “superior to monosomy 3” (data not given). Limitations of the study include the small patient population and heterogeneity of tumor types.

OncoVue® Breast Cancer Risk Test (interGenetics® Oklahoma City, OK) is a genetic-based predictive test that uses genotyping from 22 single nucleotide polymorphisms (SNPs) plus personal medical history to estimate a woman’s breast cancer risk. OncoVue was developed from the analysis of 117 genetic markers in candidate genes likely to influence breast carcinogenesis. The test uses a proprietary technology called CombiSNP®. The woman uses a mouthwash and deposits the fluid into a test tube. DNA cells from the mouth fluid are analyzed for multiple genes that are proposed indicators of breast cancer risk. The test results and the patient’s medical history are combined to give the woman her risk (standard, moderate or high risk) of developing breast cancer when she is pre-menopausal, menopausal and post-menopausal. OncoVue is recommended for patients aged 30–69 years and is a once-in-a lifetime test. The test is not intended for the diagnosis, prediction or detection of response to therapy, or to help select the optimal therapy for patients. Per InterGenetics, the test is > 99% accurate (InterGenetics, 2011). There is insufficient evidence in the published peer reviewed literature to support the accuracy and clinical utility of OncoVue.

The Oncotype DX Prostate Cancer ASSAY (Genomic Health, Redwood City, CA) is a multi-gene RT-PCR assay proposed to help determine the most appropriate treatment options. The most recent tissue sample (needle biopsy) is tested to algorithmically calculate the Genomic Prostate Score (GPS) to determine how likely the cancer is to be low risk or higher risk. The test measures the expression of 17 genes: FAM13C, KLK2, AZGP1, SRD5A2, BGN, COL1A1, SFRP4, ARF, ATP5E, CLTC, GPS1, PGK1, FLNC, GSN, TPM2, GSTM2, TPX2. Twelve genes are cancer related and five are reference genes. Oncotype DX is indicated for a man recently diagnosed with low- or intermediate-risk prostate cancer and hasn’t begun treatment. The results of the test are used in combination with the Gleason score and other clinical information (Genomic Health, 2013, Agency for Healthcare Research and Quality [AHRQ], 2013, Knezevic, et al, 2013). There is insufficient evidence to support the clinical utility of Oncotype DX for prostate cancer.

Oncotype DX was analytically and clinically validated in a study by Knezevic et al. (2013). Analytical accuracy was reported as "excellent" with average biases at qPCR inputs representative of patient samples < 9.7% across all assays. Amplification efficiencies were within ± 6% of the median. Ten prostate cancer RNA samples were tested for reproducibility and precision using multiple instruments, reagent lots, operators, days (precision), and RNA input levels (reproducibility) and appropriately parameterized linear mixed models.

Multiprotein Panel Testing for the Detection of Ovarian Cancer in a Pelvic Mass: Diagnostic studies have been introduced that are proposed to aid in the surgical decision of a woman with a pelvic mass. The tests are indicated to help determine if the tumor is benign or malignant and who should perform the surgery.

OVA1 (Vermillion, Inc., Fremont, CA) is an FDA 510(k)-approved in vitro diagnostic multivariate index (IVDMIA) test performed on serum and includes five immunoassay biomarkers: transthyretin (TT or prealbumin), apolipoprotein A-1 (Apo A-1), beta2-microglobulin (beta2M), transferrin (Tfr) and cancer antigen 125 (CA 125 II). Using a proprietary software algorithm (OvaCalc®), a numerical score of 0–10 is produced from the results of all five markers to help determine if a pelvic mass is benign or malignant. Based on a high sensitivity and negative predictive value, the test is proposed to aid the physician in determining what type of surgery should be done and by which specialist. OVA1 is intended for use in women age 18 years and older who have an ovarian adnexal mass for which surgery is planned, but prior clinical and radiological evaluation does not indicate a malignancy. The test should not be used alone, is not recommended for screening or diagnosing ovarian cancer, and should not be used in an individual with a diagnosis of malignancy within the past five years (FDA, 2009; Quest Diagnostics, 2011). There is insufficient evidence in the published, peer-reviewed literature to establish the clinical utility of OVA1.

Bristow et al. (Aug 2013) conducted a retrospective review of data from two prospective studies to determine the impact of using OVA1, CA125, Dearking modified ACOG referral guidelines and clinical assessment (physical examination, family history, imaging, laboratory) for patients (n=770) with an adnexal mass who were undergoing surgery and were initially assessed by a nongynecologic oncologist. Reported outcomes included the following:

- Overall prevalence of malignancy was identified in 164 patients (21.3%)
- triage based on CA125 predicted 157 referrals (sensitivity of 68.3%)
- triage based on modified-ACOG guidelines predicted 256 referrals (sensitivity 79.3%)
- clinical assessment predicted 184 referrals (sensitivity 73.2%)
- nongynecologic oncologists referred 462 patients to a gynecologic oncologist for surgery
- risk stratification of OVA1 would have resulted in 429 referrals (sensitivity of 90.2%)
- statistically significant higher sensitivity (p<0001) and lower specificity (p<0001) for detecting malignancy were seen with OVA1 compared to clinical assessment, CA125, and modified-ACOG guidelines

Author-noted limitations of the study included: retrospective study design; potential patient selection bias; observer bias as physicians were enrolled based on referral patterns; data on enrolling provides was based on basic specialty-specific information and the possible impact of individual provider characteristics on referral patterns is unknown; and based on the context of data collection, the outcomes may not be generalizable to all practice settings. These finding do not represent a lower risk patient population who were not referred for surgery.

Bristow et al. (Feb 2013) conducted a prospective case series to validate the effectiveness of OVA1 in identifying ovarian malignancy compared to clinical assessment plus CA125-II in 494 women scheduled for surgery for an adnexal mass by a non-gynecological oncology provider. Clinical assessment included physical examination, family history, imaging, laboratory tests and pre-surgical prediction of malignancy. A total of 92 patients (18.6%) had a pelvic malignancy. Primary ovarian cancer was diagnosed in 65 patients (13.2%), of which 43.1% were FIGO stage I disease. When OVA1 results were combined with clinical assessment, the sensitivity was 95.7%. OVA1 predicted ovarian malignancy in 91.4% of early-stage disease compared to 65.7% by CA125-II and identified 83.3% malignancies missed by clinical impression and 70.8% missed by CA125-II. Negative predictive value was 98.1% in predicting the absence of ovarian cancer. Clinical impression and CA125-II were more accurate at identifying benign disease. Combined with clinical impression, OVA1 correctly identified 204 benign cases. Author noted limitations of the study included: inclusion criteria required subjects who were planned to undergo surgery for adnexa mass which represents a higher prevalence of pelvic malignancy that would not be expected in patients not scheduled for surgery; inability to interrogate the process

of physician assessment; and finally, the study was not “designed to address the impact on referral patterns of the lower level of specificity with OVA1, which could lead to referral of a higher proportion of patients with non-malignant tumors”.

In a study submitted to the FDA in which patients (n=269) were treated by non-gynecologic oncologists, results of OVA1 were compared to pathology reports from biopsied tissue. Overall, pre-surgical assessment (i.e., single assessment) revealed a sensitivity of 61.0%–81.2%, specificity of 76.9%–87.4%, positive predictive value of 49.9%–70.1%, and a negative predictive value of 83.7%–92.8%. With dual assessments (i.e., pre-surgical and OVA1 assessment), the values included: sensitivity of 83.0%–96.1%, specificity of 35.0%–48.6%, positive predictive value of 29.8%–43.7%, and negative predictive value of 85.9%–96.8%. Sensitivity was as high as 96% in postmenopausal women. The prevalence of malignancy among patients with adnexal mass assessed by non-gynecologic oncologists was 26.8% (FDA, 2009).

In a multicenter prospective study, Ueland, et al., (2011) compared the effectiveness of physician assessment (n=516) to OVA1 (n=524) and CA 125 (n=524) in identify high risk ovarian tumors. Final pathology identified 161 malignant and 363 benign ovarian tumors. The study included recruited women, ages 18 years and older, with an ovarian tumor (pathology diagnosis varied), and planned surgical intervention within three months of radiological imaging. The sensitivity of OVA1 was statistically superior to physician assessment ($p<0.001$), CA 125 with a cutoff of 67 units/milliliter (ml) and CA 125 with a cutoff of 200 units/ml ($p<0.001$ each). The sensitivity of OVA1 for epithelial ovarian cancer was 99% (93/94), nonepithelial ovarian cancer 82% (9/11), borderline ovarian tumor 75% (21/28), and metastases to ovary 94% (17/18). Overall, statistical analysis revealed the following:

	Sensitivity (%)	Specificity (%)	Positive Predictive Value (PPV) (%)	Negative Predictive Value (NPV) (%)
CA 125 (67 units/mL)	77	73	56	88
CA 125 (200 units/mL)	69	84	65	86
OVA1	93	43	42	93
Non-gynecologic oncologist assessment alone	72	83	60	89
Non-gynecologic assessment plus OVA1	92	42	36	93
Gynecologic oncologist alone	78	75	63	86
Gynecologic oncologist plus OVA1	99	26	43	98
All physician assessment	75	79	62	88
All physician assessments plus OVA1	96	35	40	95

OVA1 plus physician assessment identified 70% (14/20) of malignancies missed by nongynecologic oncologists and 95% (19/20) of malignancies missed by gynecologic oncologists. A total of 261 of 363 (72%) patients with benign tumors were referred to gynecological oncologist for surgery. Non-gynecological oncologists referred 83 of 183 (45%) benign tumors to gynecological oncologist for surgery. Physician assessment plus OVA1 identified malignancies missed by physician assessment in 70% of nongynecologic oncologists, and 95% of gynecologic oncologists. OVA1 detected 76% of malignancies missed by CA 125. Physician assessment plus OVA1 identified 86% of malignancies missed by CA 125. Results varied based on whether the woman was premenopausal or menopausal and the type of cancer. A potential limitation of the study noted by the authors is the “lack of a uniform preoperative evaluation for comparison.” Other limitations are the heterogeneous patient population and lack of randomization. There was no discussion regarding how patients were treated as a result of the OVA1 results.

Ware-Miller et al. (2011) conducted a prospective multi-center study to compare the performance of the American College of Obstetricians and Gynecologists (ACOG) published referral guidelines for women (n=516) with a pelvic mass to the performance of the ACOG guidelines using CA 125 compared to using OVA1. The ACOG referral guidelines incorporated menopausal status, physical examination, family history, imaging and CA 125. The results of a standard CA 125-II assay were applied to the OVA1 algorithm and the CA 125 analysis.

The results were then correlated with surgical pathology. The study included recruited women, ages 18 years and older, diagnosed with an ovarian tumor with planned surgery within three months of radiologic imagining. Of the 516 patients, 161 had a malignancy. For all patients, the performance of the ACOG guideline with the Dearking modification (i.e., "eliminating the family history of one or more first-degree relatives with ovarian or breast cancer, and lowering the CA 125 threshold in premenopausal women to 67 units/mL") was not significantly different from the ACOG criteria. When separated by menopausal status, the modified ACOG guidelines were associated with an increase in sensitivity from 58% to 76% and a decrease in specificity from 77% to 70% for premenopausal women, and an increase in specificity from 56% to 71% for postmenopausal women. On univariate analysis, the highest odds ratio for predicting ovarian cancer was seen using CA 125, presence of ascites and radiographic evidence of metastasis. When the OVA1 results were substituted for CA 125 results in the ACOG guidelines, the sensitivity of the guidelines was significantly higher ($p<0.001$). The NPV increased and the specificity and PPV decreased. Results varied when calculations were done by subgroups (i.e., premenopausal, postmenopausal and cancer type). When all 161 malignancies were evaluated, the ACOG guidelines with OVA1 identified 15 of 19 (79%) missed malignancies in premenopausal women and 12 of 18 (67%) malignancies missed in postmenopausal women compared to ACOG guidelines. For primary epithelial and nonepithelial ovarian cancers, the ACOG guidelines plus OVA1 identified seven of nine (78%) missed early-stage premenopausal malignancies and five missed postmenopausal malignancies. The ACOG guidelines plus OVA1 also identified 25 of 27 (93%) premenopausal and 76 of 76 postmenopausal primary ovarian malignancies. The authors noted that "beyond identifying more malignancies, it is not known precisely how the multivariate index assay will affect the referral of patients." A potential limitation of the study noted by the authors was "the use of the newer CA 125-II assay rather than the original assay". Other limitations include: the guidelines were evaluated by a diverse group of primary care and specialty centers, and the heterogeneous patient population. The impact of OVA 1 on patient management was not reported.

In their practice guidelines for ovarian, fallopian tube, and primary peritoneal cancers, NCCN (2013) notes that the Society of Gynecologic Oncologists and the FDA do not recommend OVA1 as a screening tool to detect ovarian cancer, but agree that all patients should undergo surgery by an experienced gynecologic oncologist.

In a 2011 Committee Opinion document discussing the early detection of epithelial ovarian cancer, the American College of Obstetricians and Gynecologists discussed the indications for OVA 1 and stated that the clinical utility of the test has not been established.

The Society of Gynecologic Oncologists' statement regarding OVA1 (2009) stated that the test is proposed to be a useful took in identifying women who should be referred to a gynecologic oncologist for ovarian cancer surgery. SGO went on to state "this test has not been approved for use as an ovarian cancer screening tool, nor has it been proven to result in early detection or reduce the risk of death from this disease".

ROMA (Risk of Ovarian Malignancy Algorithm) (Fujirebio Diagnostics Inc., Malvern, PA) combines the woman's menopausal status with the CA 125 and HE4 values to report a risk of epithelial ovarian cancer. The test calculates a risk of finding ovarian cancer during surgery and classifies the patient as low risk or high risk for malignant disease. According to the manufacturer, at a set specificity of 75%, the sensitivity for epithelial ovarian cancer and low malignant potential tumors was 89% with a 94% negative predictive Value (NPV) for pre and post menopausal women. Using the HE4 assay and the ARCHITECT CA125II the ROMA value for high risk of finding epithelial ovarian cancer was ≥ 13.1% and < 13.1% low risk of finding cancer. For postmenopausal women, the Roma value was ≥ 27.7% for high risk and < 27.7% low risk.

Van Gorp et al. (2012) conducted a single-center, prospective cohort study to evaluate the outcome of greyscale and color Doppler ultrasound, risk of malignancy index (RMI) and ROMA in 432 consecutive women with a pelvic mass of suspected ovarian origin who were scheduled for surgery. A total of 374 patients were analyzed, of which 224 had benign disease and 150 had malignant disease. Subjective ultrasound assessment had the highest area under the receiver operator characteristic curve (AUC) (0.968), followed by the risk of malignancy index (RMI) (0.931). Both the subjective ultrasound assessment ($P<0.0001$) and RMI ($p=0.0030$) had significantly higher AUCs than ROMA. There were no significant differences in pre- and postmenopausal women. The results of this study suggested that subjective ultrasound assessments were superior to RMI and ROMA. The authors noted that limitations of the study included the fact that 13.4% of eligible patients with a malignant adnexal mass were excluded and the study setting was a tertiary center with a specialized gynecological ultrasound unit.

Molina et al. (2011) conducted a prospective validation study to evaluate HE4 and CA 125 in healthy subjects (n=66) and patients with benign (n=285) and malignant gynecological diseases (n=143 with active cancer; n=33 without active disease after radical treatment). Benign diseases included ovarian cysts, myomas, endometriosis, endometrial polyps and other diseases. Malignant diseases included endometrial cancer, endocervical cancer, squamous cell carcinoma of the cervix and ovarian cancer. Women were premenopausal and postmenopausal. Significantly higher CA 125 levels were found in premenopausal women compared to postmenopausal women ($p=0.001$). In healthy women HE4 was abnormal in 1.5%, CA 125 in 13.6% and ROMA 25.8% compared to 1.1%, 30.2% and 12.3% in women with benign diseases, respectively. Compared to CA 125, HE4 had a significantly higher concentration in ovarian cancer (n=222) ($p<0.001$). In ovarian cancer the sensitivity for HE4 was 79.3%, 82.9% for Ca 125 and 90.1% for ROMA. HE4 and CA 125 were related to tumor stage and histology and had their lowest concentration in mucinous tumors. Compared to CA 125, a significantly higher area under the ROC curve was obtain with ROMA and HE4 in differential diagnosis of benign disease vs. malignant disease. The authors proposed that the data from this study indicated that the ROMA algorithm might be further improved if it was used only in patients with normal HE4 and abnormal CA 125 levels and that HE4 was the marker of choice in ovarian cancer due to its higher sensitive in early stages and it specificity and efficiency over CA 125 and ROMA. A limitation of the study is the heterogeneous patient population.

In a prospective study, Kim et al. (2011) recruited 159 women with an adnexa mass (n=78 with ovarian cancer; n=81 with benign disease) and 224 women as health controls to evaluate the serum levels of HE4 and CA 125 and utilized ROMA to categorize ovarian patients into low and high risk. HE4 and CA 125 were significantly higher in patients with ovarian cancer compared to patients with benign disease and healthy controls ($p<0.0001$, each). HE4 was less affected by benign conditions than CA 125. The area under the ROC curve was higher when both markers were used compared to CA 125 only. ROMA successfully classified ovarian patients into high and low risk with 87.5% sensitivity and 93.8% specificity.

Jacob et al. (2011) conducted a prospective study to compare the results of HE4, CA 125 and ROMA values in 33 healthy controls, 72 patients with benign disease and 56 tumor patients. Healthy women and women with benign disease had low levels of HE4 and CA125. HE4 was not elevated in endometriosis. HE4 and ROMA had the best diagnostic performance when benign disease (n=71) was compared to early stage ovarian and tubal cancers (n=19) (specificity 85.9% vs. 87.3% and sensitivity 78.9% vs. 78.9% respectively). CA 125 detected peritoneal cancer better than HE4 and ROMA with a specificity of 97.2% and a sensitivity of 80.0%. The authors noted that HE4 had a better detection rate in borderline tumors and early state ovarian and tubal and better results than CA 125 with or without ROMA and did not see the benefit of combining CA 125 and HE4.

In a retrospective review, Ruggeri et al. (2011) compared the results of HE4 (using two different methods), CA 125 and ROMA in 73 healthy women, 90 women with benign ovarian or adnexal diseases and 96 women with epithelial ovarian cancer (EOC). HEA sensitivity ranged from 79.2% to 89.6% compared to 59.4% to 86.5% with CA 125. The overall sensitivity of ROMA was 95.8% at 75% specificity compared to 87.1% to 87.6% for HE4 plus CA 125.

In their 2013 clinical practice guidelines on ovarian cancer, NCCN stated that although human epididymis protein 4 (HE4) appears to be useful in detecting ovarian cancer, recent data showed that several markers, including HE4, do not increase early enough to be useful in detecting early-stage ovarian cancer.

OPN (Osteopontin): OPN is a secreted adhesive glycoprotein, originally isolated from bone extracellular matrix and proposed as a potential marker for metastatic uveal melanoma. An early pilot study (n=27) reported statistically significant differences in the levels of OPN in patients with metastases compared to patient without metastases (Reiniger, et. al., 2007). There is insufficient evidence to support the use of OPN as a marker for uveal melanoma.

P53 (Monoclonal Antibody): P53 has been proposed for use in prognosis and prediction of recurrence and disease-free survival of breast, prostate, colorectal, gastric and bladder cancer. Although researchers have used immunohistochemical assays in an attempt to determine if the detection of P53 could be a useful marker, their findings have been inconclusive (ASCO, 2006; Pister, et al., 2005).

Regarding bladder cancer NCCN (2013) stated that P53 is still considered experimental. In practice parameters for anal squamous neoplasms, the American Society of Colon & Rectal Surgeons (ASCRS) (2012) stated that biomarkers such as tumor suppressor genes P53 and P21 have shown promise but they have a limited role in follow-up of these patients.

P-LAP (Placental Alkaline Phosphatase): PLAP was first identified in patients with lung cancer and later in other cancers. PLAP has been proposed as a marker for testicular cancer and "is detected in most seminomas and embryonal carcinomas, in 50% of yolk sac tumors and choriocarcinomas, but only rarely in teratomas". PLAP levels may be increased up to 10-fold in smokers. This and the paucity of commercial assays limit its clinical application and serum assays for P-LAP are not routinely included in the diagnostic work up of testicular cancer patients (NACB, 2009).

PSMA (Prostate-Specific Membrane Antigen): PSMA has been proposed as a marker for the monitoring of prostate cancer, but has not been proven to be better than PSA (ACS, 2011).

S-100: S-100 is a protein that can be found in most melanoma cells. Tissue samples may be tested for this marker to help in diagnosing melanoma metastasis (ACS, 2011). Additional research of this protein is needed to determine its accuracy in being used as a tumor marker.

SCC-Ag (Squamous Cell Carcinoma Antigen): SCC-Ag has been proposed as a possible serum tumor marker to be used for the detection of various types of squamous cell tumors, including non-small cell lung cancer, cervical cancer, and esophageal cancer. This marker may also be referred to as tumor-associated antigen (TA-4) because SCC-Ag is thought to be a part of this larger molecule. Studies conducted to date on this antigen have not proven its efficacy on patient morbidity and mortality. Sensitivity and specificity have not been analyzed to identify which patient population would benefit from the use of this marker in determining prognosis, treatment planning and/or follow-up. A variety of nonmalignant disease can result in an elevated SCC-ag, therefore it should not be used alone to diagnose the presence of a malignancy (NACB, 2009)

SLEX (Sialyl Lewis X Antigen): SLEX is proposed to be expressed in liver disease, and hepatocellular carcinomas. The expression of SLEX by cancer of an epithelial origin correlates with metastases and poor prognosis (Varki, et al., 2008; Malagolini et al., 2007; Bachner, 2005). There is a lack of evidence to support the use of SLEX as a tumor marker in the management of carcinomas.

SLX (Sialyl X): SLX is a carbohydrate antigen adhesion molecule on the cell surface of adenocarcinomas. It has been reported to be elevated in many types of carcinoma including advanced non-small lung cancer or in the presence of metastasis. In gastric and colon cancer, elevated levels of SLX have been associated with lymph node and distant metastasis, but its clinical utility has not been established (Mizuguchi, et al., 2007).

Systems Pathology for Predicting Risk of Recurrence in Prostate Cancer: Microarrays called "systems pathology" have been proposed for predicting cancer recurrence in patients with prostate cancers. These tests include molecular and imaging data with clinical and pathological information to risk stratify patients' outcomes. There is insufficient evidence in the published peer-reviewed literature to support the accuracy and clinical utility of these tests (Donovan, et al., 2009).

Prostate PX+ (Aureon® Biosciences, Yonkers, NY) is a systems pathology prognostic test that analyzes a patient's prostate biopsy tissue along with clinical data to assist the physician in determining treatment options. The test provides a PX+ Score™ and information that is proposed to identify low-risk patients with aggressive disease, stratify intermediate-risk patients into low or high risk, assess favorable pathology and provide a recurrence score. The report also includes a disease progression score, quantitative morphometric features, photomicrographs and biomarkers. The Aureon technology includes the PathoMetrix™, Aureon M-Plex™, and Discovery-Path™. Prostate PX+ was developed in a retrospective clinical trial (n=686) using clinical-pathological, morphometric and molecular data and validated in an external cohort (n=341). The use of the test is recommended at the time of the diagnosis of prostate cancer. The test is performed in a CLIA laboratory (Aureon Biosciences, 2011).

Aureon also offers a Post-Op Px® prognostic test that is recommended for use following a prostatectomy. This test is proposed to provide a PSA recurrence score reflective of the biochemical recurrence within five years of

surgery and to predict disease progression by evidence of bone/soft tissue metastasis and progression through hormonal therapy within five years of surgery. The Post-Op Px test combines information from histology, molecular markers and clinical information to predict recurrence and disease progression. The test is based on data from a study of 758 patients and is performed in a CLIA laboratory (Aureon Biosciences, 2011). Current information indicates that this laboratory is no longer operational.

In their prostate cancer clinical guidelines, NCCN (2013) stated that molecular markers are being investigated as a tool to further refine patient's risk of recurrent cancer, but these "approaches remain investigational and are not available currently or validated for routine application."

TA-90 (Tumor Antigen 90): TA-90 is a protein that is found on the outer surface of melanoma cells and has been proposed for the detection of metastasis of melanoma cells. TA-90 has also been proposed for use in colon and breast cancers but its value is undetermined and it is not widely used at this time.

TATI (Tumor-Associated Trypsin Inhibitor): Elevated levels of this inhibitor have been found in patients with various cancers including gynecological and pancreatic. Because of TATI's low sensitivity it is not recommended for monitoring disease (NACB, 2009).

TNF-a (tumor necrosis factor alpha): TNF plays a significant role in inflammation and immune response and is found in the serum of cancer patients, rheumatoid arthritis, infections and acquired immunodeficiency syndrome (AIDS) (Tisdale 2008; Elghetany and Banki, 2006). The clinical utility of this marker in the management of cancer patients has not been established.

TPA (Tissue Polypeptide Antigen): This protein marker has been found in high levels in patients with lung, liver, bladder, and many other cancers. TPA is not an established tumor marker because it is not specific to one particular cancer and is also elevated in noncancerous conditions (NACB, 2010; NACB, 2009).

Tumor Profiling: Caris Target Now™ Comprehensive (Caris Life Sciences™, Dallas TX) is a tumor analysis coupled with a clinical literature search to identify appropriate therapies for patients based on biomarkers. The test is performed after a cancer diagnosis has been made and the patient has exhausted all standard therapies and questions regarding therapeutic management exist. The analysis is proposed to identify biomarkers that may have an influence on therapy and treatment regimens for the patient are suggested by Caris Life Sciences. The test begins with tumor analysis by immunohistochemistry (IHC) for cancer cell proteins and if the sample is frozen, a microarray analysis may be performed. Based on the results of IHC and microarray, Caris Life Sciences may perform fluorescent in-situ hybridization (FISH) to examine gene copy number variations in the tumor and polymerase chain reaction (PCR) and/or DNA sequencing to determine gene mutations in the tumor tissue. It is up to the discretion of Caris Life Sciences™ as to which test(s) should be run based on "published findings" from cancer researchers. The analysis provides potential therapies for patients to discuss with their physician. Caris Life Sciences is a Clinical Laboratory Improvement Amendments (CLIA) certified laboratory. IHC testing includes the following: androgen receptor, BCRP, COX-2, CAV-1, CD20, CD52 (for hematologic malignancies only), c-kit, CK 5/6, CK 14, CK 17, Cyclin D1, E-Cad, ER, ERCC1, Her2/neu, Ki67, MGMT, MRP1, p53, p95, PDGFR, PGP, PR, PTEN, RRM1, SPARC, TLE3, TOP2A, TOPO1, TS. Mutational analysis by targeted sequencing includes KRAS: exons 2 & 3; BRAF: exons 11 & 15; c-Kit: exons 9 & 11 adding 13 & 17; EGFR kinase domain sequencing: exons 18–21; and PIK3CA: exons 9 & 20. Mutational analysis by PCR is performed for KRAS and FISH testing includes ALK, HER2, EGFR, cMyc, and TOP2A. Focused molecular profiling for specific cancers (e.g., breast, colorectal, NSCLC, melanoma, ovarian surface, other solid tumors) are also available through the Caris Target Now Select profiles (Caris Life Sciences, 2013). There is insufficient evidence in the published peer-reviewed literature to support the accuracy and clinical utility of these diagnostic profiles.

Von Hoff et al. (2010) conducted a multicenter prospective study (n=86) to compare progression-free survival (PFS) of a patient's tumor using therapy selected by molecular profiling (MP) (Caris Life Sciences) to PFS for the most recent therapy on which the patient experienced disease progression. They also evaluated the frequency with which MP by IHC/FISH and microarray yielded a target against a commercially available therapeutic agent and determined the response rate and patients without progression or death at four months. Out of the 86 patients who proceeded with MP, 66 were treated as a result of MP. Of the 66 treated, the cancer types included the following: breast (n=18); colorectal (n=11); ovarian (n=5); prostate (n=4); lung (n=3); two

patients each with melanoma, small cell esophageal/retroperitoneal, mesothelioma, head and neck, and pancreas; and one each cholangiocarcinoma, pancreas neuroendocrine, unknown primary, gastric, duodenal, peritoneal pseudomyxoma, anal canal, vaginal, cervix, renal, eccrine sweat adenocarcinoma, salivary gland adenocarcinoma, soft tissue uterine sarcoma, gastrointestinal stromal tumor (GIST) and thyroid anaplastic. Eighteen patients had a PFS ratio ≥ 1.3 and therefore, the null hypothesis was rejected and the MP approach was considered "promising" by the authors ("null hypothesis was that ≤ 15% of the patient population would have a PFS ratio of ≥1.3"). The assessment included tumor analysis, imaging, clinical evaluation and laboratory testing. A total of eight breast cancer patients, four colorectal cancers, five ovarian cancer and five of the other miscellaneous cancers (the one and two types listed above) had a PFS ratio ≥ 1.3. Fourteen patients were treated with suggested combination therapies and four with single agents, some of which involved off-label usage. Regarding the secondary end points, MP yielded a target in 84 patients compared to 83 by IHC/FISH and 81 by microarray. Using the Response Evaluation Criteria in Sold Tumors (RECIST), one complete response was seen in a breast cancer patient and five partial responses were seen in two breast cancer patients and one each ovarian cancer, rectal cancer and non-small-cell lung cancer for an overall response rate of 10%. At four months, 14 of the 66 patients were without progression. Nine "serious" adverse events were reported (e.g., anemia, neutropenia, dehydration, pancreatitis). Author-noted limitations of the study included the following: "not a lot of experience exists with PFS ratio (patients as their own controls) as a clinical endpoint" which can introduce bias; lack of randomization; patient attrition rate (66 our of 106 evaluated initially for the study were treated based on MP); and the "selection of a commercially available agent to suggest for treatment against a particular target was based on an extensive literature review and prior experience in a feasibility study" which included retrospective reviews. Finally, the authors stated that "there is a tremendous need for additional prospective evaluation of these biomarkers to determine response or lack of response"

Use Outside of the US

In a guideline for biomarker testing in colorectal cancer, the Spanish Society of Pathology (SEAP) and the Spanish Society of Medical Oncology (SEOM) stated that although Oncotype DX-Colon has been shown to have prognostic value, there is no consensus on its use in clinical practice. The authors noted that the clinical usefulness of the test was compromised because the predictive value of Oncotype DX could not be validated (Garcia-Alfonso, 2012).

The 2010 National Institute for Health and Clinical Excellence (NICE), United Kingdom, guidance document on the diagnosis and management of carcinomas of unknown primary (CUP) recommended against the use of gene-expression-based profiling (e.g., Pathwork Tissue of Origin) to identify primary tumors in patients with provision CUP. According to NICE, there is limited evidence that gene-expression profiling changes the management of the patient, and there is no evidence of improvement of outcomes.

The Australia and New Zealand Horizon Scanning Networks (ANZHSN) program is a collaborative Commonwealth and State initiative guided by the Health Policy Advisory Committee on Technology (HealthPACT). A HealthPACT technology summary on diagnostic tests for ovarian cancer (2010) stated that OvPlex™ (HealthLinx Ltd, Australia) is a test available directly to the consumer for the proposed purpose of providing early detection of ovarian cancer. OvPlex includes five biomarkers including CA-125, C-reactive protein (CRP), serum amyloid A (SAA), interleukin 6 (IL-6) and interleukin 8 (IL-8) and uses an algorithm to analyze the concentration of the biomarkers. The test is similar in concept to the OVA1 in the US. The authors noted that the available studies for OvPlex were in the "proof of concept state" because the sensitivity and specificity have been calculated on a high risk population. Health PACT concluded that "based on the poor quality of evidence of studies conducted in inappropriate populations, and in light of ethical concerns and the potential to do harm associated with this direct-to-consumer test, it is recommended that this summary be disseminated to CTEPC, consumer health groups, the College of General Practitioners and the National Breast and Ovarian Cancer Centre".

Cancer screening clinical practice guidelines by the Ministry of Health (MOH), Singapore (2010), stated that the use of serum markers for the screening in women at average risk for epithelial ovarian cancer is not recommended, the use of biomarkers as a screening tool for lung cancers is under investigation and there is currently no role for biomarkers other than PSA for primary screening for prostate cancer.

Summary

Recommendations and guidelines by professional societies and organizations and evidence in the published peer-reviewed scientific literature support the use of defined tumor makers for the screening, diagnosis, treatment planning, treatment-monitoring and/or follow-up of specific cancers.

However, improvements in meaningful health outcomes for numerous other tumor markers have not been proven and they are still under investigation to determine their clinical utility in the management of individuals with various types of cancers. Overall, clinical trials have primarily been in the form of retrospective validation studies with small heterogeneous patient populations and short-term follow-ups. Studies comparing the tumor markers to established treatment options are lacking and management of patients based on the results of these markers has not been published.

Appendix A
Tumor Marker(s) by Cancer Type Covered as Medically Necessary

Cancer Type	Tumor Marker(s)
Acute myeloid leukemia	MPO
Bladder	BTA, ImmunoCyte/yCyte+, NMP22, UroVysion
Breast	CA 15-3, CA 27.29, BR 27.29 or Truquant RIA, CEA, ER/PR, HER2
Carcinoid tumors	5-HIAA
Colorectal	CEA
Endometrial	CA-125
Gallbladder	None Indicated
Gastrointestinal Stromal Tumors	C-kit, CD-117
Gastric or Esophagogastric Junction	HER2
Hepatocellular, Primary	AFP
Lung, Small Cell	NSE
Multiple Myeloma	B_2M
Myeloid Leukemia, Acute	MPO
Neuroendocrine (e.g., carcinoid tumors, neuroblastoma, and small cell lung cancer)	CgA
Ovarian, Epithelial	CA-125
Ovarian, Germ Cell	AFP with b-HCG
Ovarian, Trophoblastic	HCG
Pancreatic	CA 19.9
Pelvic Mass, Undiagnosed	AFP with b-HCG, CA-125
Prostate	PSA*
Gastric or Esophagogastric Junction	HER2
Testicular, Nonseminoma Germ Cell	AFP with b-HCG
Testicular Trophoblastic	HCG
Thyroid, Medullary	Calcitonin, CEA
Thyroid, Differentiated	Thyroglobulin
Thyroid, Indeterminate Nodule	Afirma® Thyroid FNA Analysis

***Refer to Cigna Coverage Policy Prostate-Specific Antigen (PSA) Screening for Prostate Cancer**

Coding/Billing Information

Note: 1) This list of codes may not be all-inclusive.
2) Deleted codes and codes which are not effective at the time the service is rendered may not be eligible for reimbursement.

Covered when medically necessary:

Tumor Markers

Marker	Covered Indication(s)	CPT®*/ HCPCS Code	Code Description
AFP	Primary hepatocellular cancer	82105	Alpha-fetoprotein; serum
AFP in combination with b-HCG (beta-human chorionic gonadotropin)	Nonseminoma germ cell testicular cancer Germ cell ovarian cancer Undiagnosed pelvic mass	82105 84702 84703 84704	Alpha-fetoprotein; serum Gonadotropin, chorionic (hCG); quantitative Gonadotropin, chorionic (hCG); qualitative Gonadotropin, chorionic (hCG); free beta chain
B_2M (beta$_2$-microglobulin)	Multiple myeloma	82232	Beta-2 microglobulin
Bladder-tumor associated antigen (BTA)	Bladder cancer	86294	Immunoassay for tumor antigen, qualitative or semiquantitative (eg, bladder tumor antigen)
Calcitonin	Thyroid medullary carcinoma	82308	Calcitonin
CA (cancer antigen) 15-3, CA 27.29, BR 27.29 or Truquant RIA	Metastatic breast cancer	86300	Immunoassay for tumor antigen, quantitative; CA 15-3 (27.29)
CA 19.9	Pancreatic Cancer	86301	Immunoassay for tumor antigen, quantitative; CA 19-9
CA 125	Epithelial ovarian cancer Endometrial cancer Undiagnosed pelvic mass	86304	Immunoassay for tumor antigen, quantitative; CA 125
CEA (carcinoembryonic antigen)	Colorectal cancer Medullary thyroid cancer Metastatic breast cancer	82378	Carcinoembryonic antigen (CEA)
CgA (chromogranin A)	Neuroendocrine tumors (e.g., carcinoid tumors, neuroblastoma, and small cell lung cancer)	86316 Note: Covered as medically necessary when used to report the Chromogranin A (CgA)	Immunoassay for tumor antigen, other antigen, quantitative (eg, CA 50, 72-4, 549), each
C-kit (CD-117 [cluster of differentiation-117])	Gastrointestinal stromal tumors	81404	Molecular pathology procedure, Level 5 (eg, analysis of 2-5 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 6-10 exons, or characterization of a dynamic mutation disorder/triplet repeat by Southern blot analysis)

Marker	Covered Indication(s)	CPT®*/ HCPCS Code	Code Description
		88342	Immunohistochemistry (including tissue immunoperoxidase), each antibody
ER/PR (estrogen receptors and progesterone receptors)	Breast Cancer	88342 88360 88361	Immunohistochemistry (including tissue immunoperoxidase), each antibody Morphometric analysis, tumor immunohistochemistry (eg, Her-2/neu, estrogen receptor/progesterone receptor), quantitative or semiquantitative, each antibody; manual Morphometric analysis, tumor immunohistochemistry (eg, Her-2/neu, estrogen receptor/progesterone receptor), quantitative or semiquantitative, each antibody, using computer-assisted technology.
5-HIAA (5-hydrocyindoleasceti c acid)	Carcinoid tumors	83497	Hydroxyindolacetic acid, 5-(HIAA)
Gene expression classifier for thyroid nodule (i.e., Afirma® Thyroid FNA Analysis)	Cytologically indeterminate thyroid nodule	81479 Note: Covered when medically necessary and used to report Gene expression classifier for thyroid nodule (i.e., Afirma® Thyroid FNA Analysis).	Unlisted molecular pathology procedure
HCG (human chorionic gonadotropin)	Trophoblastic testicular cancer Trophoblastic ovarian cancer	84702 84703 84704	Gonadotropin, chorionic (hCG); quantitative Gonadotropin, chorionic (hCG); qualitative Gonadotropin, chorionic (hCG); free beta chain
HER2 (human epidermal growth factor receptor 2) when performed by immunohistochemic al (IHC) and/or fluorescent in situ hybridization (FISH) ImmunoCyte™/yCyt e+™	Breast cancer	83950 88271 88342 88360 88361	Oncoprotein, HER-2/neu Molecular cytogenetics; DNA probe, each (eg, FISH) Immunohistochemistry (including tissue immunoperoxidase), each antibody Morphometric analysis, tumor immunohistochemistry (eg, Her-2/neu, estrogen receptor/progesterone receptor), quantitative or semiquantitative, each antibody; manual Morphometric analysis, tumor

Marker	Covered Indication(s)	CPT®*/ HCPCS Code	Code Description
			immunohistochemistry (eg, Her-2/neu, estrogen receptor/progesterone receptor), quantitative or semiquantitative, each antibody; using computer-assisted technology.
MPO (myeloperoxidase)	Acute myeloid leukemia	83876	Myeloperoxidase (MPO)
Nuclear-Matrix Protein (NMP22)	Bladder cancer	86386	Nuclear Matrix Protein 22 (NMP22), qualitative
NSE (neuron-specific enolase)	Small cell lung cancer	86316	Immunoassay for tumor antigen, other antigen, quantitative (eg CA 50, 72-4, 549), each
PSA (prostate-specific antigen)	Prostate cancer	84152 84153 84154 G0103	Prostate specific antigen (PSA); complexed (direct measurement) Prostate specific antigen (PSA); total Prostate specific antigen (PSA); free Prostate cancer screening; prostate specific antigen test (PSA)
Thyroglobulin	Differentiated thyroid cancer	84432	Thyroglobulin
Urovysion	Bladder cancer	88120 88121	Cytopathology, in situ hybridization (eg, FISH), urinary tract specimen with morphometric analysis, 3-5 molecular probes, each specimen; manual Cytopathology, in situ hybridization (eg, FISH), urinary tract specimen with morphometric analysis, 3-5 molecular probes, each specimen; using computer-assisted technology

Paraneoplastic (Onconeural) Antibodies (anti-Hu, anti-YO, antiCV2, anti-RI, anti-Ma2, anti-amphiphysin)

Covered when medically necessary for the evaluation of neurological symptoms when the diagnosis remains uncertain following conventional work-up and an occult neoplasm is suspected:

CPT* Codes	Description
83520	Immunoassay for analyte other than infectious agent antibody or infectious antigen; quantitative, not otherwise specified
84181	Western Blot, with interpretation and report, blood or other body fluid
84182	Western Blot, with interpretation and report, blood or other body fluid, immunological probe for band identification, each

Other Tumor Markers

Experimental/Investigational/Unproven/Not Covered for the screening, staging, diagnosis, monitoring and/or surveillance of cancer:

CPT* Codes	Description
81287	MGMT (O-6-methylguanine-DNA methyltransferase) (eg, glioblastoma multiforme), methylation

	analysis
81479†	Unlisted molecular pathology procedure
81500	Oncology (ovarian), biochemical assays of two proteins (CA-125 and HE-4), utilizing serum, with menopausal status, algorithm reported as a risk score
81503	Oncology (ovarian), biochemical assays of five proteins (CA-125, apolipoprotein A1, beta-2 microglobulin, transferring, and pre-albumin), utilizing serum, algorithm reported as a risk score
81504	Oncology (tissue of origin), microarray gene expression profiling of > 2000 genes, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as tissue similarity scores
81599†	Unlisted multianalyte assay with algorithmic analysis
82387	Cathepsin-D
83520	Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise specified
83951	Oncoprotein; des-gama-carboxy-prothrombin (DCP)
84275	Sialic acid
84999†	Unlisted chemistry procedure
88358	Morphometric analysis; tumor (eg, DNA ploidy)
	Multiple/Varied

†**Note: Not covered when used to report any tumor marker indicated in this Coverage Policy as experimental, investigational/unproven.**

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