Tumor Profiling, Gene Expression Assays and Molecular Diagnostic Testing for Hematology/Oncology Indications

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Related Coverage Resources

Genetics
Genetic Testing Collateral File

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**Coverage Policy**

Many benefit plans limit coverage of genetic testing and genetic counseling services. Please refer to the applicable benefit plan language to determine benefit availability and terms, conditions and limitations of coverage for the services discussed in this Coverage Policy.

For additional information regarding coverage for specific genetic tests please refer to the Genetic Testing-Collateral File.

**General Criteria for Somatic Mutation Genetic Testing**

**Medically Necessary**

Tumor biomarker or gene expression classifier (GEC) testing is considered medically necessary when ALL of the following criteria are met:

- The individual is a candidate for a targeted therapy associated with a specific tumor biomarker or disease site
- Results of testing will directly impact clinical decision making
- The testing method is considered to be scientifically valid and proven to have clinical utility based on prospective evidence
- EITHER of the following:
  - identification of the specific biomarker or risk assessment using a GEC is required in order to initiate a related therapy and the therapy has been validated by the National Comprehensive Cancer Network™ (NCCN Guidelines™) as a category 1, 2A, or 2B recommendation for the individual’s tumor type or disease site
  - identification of the specific biomarker or use of a GEC has been demonstrated in published peer-reviewed literature to improve diagnosis, management or clinical outcomes for the individual’s condition being addressed

**Experimental/Investigational/Unproven:**

Molecular testing for hematology-oncology indications is considered experimental, investigational or unproven in the following situations:

- there is insufficient evidence to support molecular testing for the specific tumor type or disease site
- the requested gene(s) or biomarker(s) are correlated with a known therapy, but that therapy has not been validated for the specific tumor type or disease site

**Tumor Profile/Gene Expression Classifier Testing**

**Medically Necessary**
Tumor profile/gene expression classifier testing (GEC) is considered medically necessary when the associated criteria are met for ANY of the following indications:

<table>
<thead>
<tr>
<th>Test Name</th>
<th>Cancer Type and Indication</th>
</tr>
</thead>
</table>
| MammaPrint® 70-Gene Breast Cancer Recurrence Assay  | For a woman with Stage I or Stage 2 invasive breast cancer being considered for adjuvant systemic therapy when ALL of the following criteria are met:  
  - high clinical risk of recurrence*  
  - estrogen receptor (ER)-positive/progesterone receptor (PR)-positive  
  - human epidermal growth factor receptor 2 (HER2)-negative  
  - up to three positive nodes  

<table>
<thead>
<tr>
<th>Tumor Grade</th>
<th>Nodes</th>
<th>Tumor Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well differentiated</td>
<td>None</td>
<td>3.1-5 cm</td>
</tr>
<tr>
<td></td>
<td>1-3</td>
<td>2.1-5 cm</td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>None</td>
<td>2.1-5 cm</td>
</tr>
<tr>
<td></td>
<td>1-3</td>
<td>Any size</td>
</tr>
<tr>
<td>Poorly differentiated or undifferentiated</td>
<td>None</td>
<td>1.1-5 cm</td>
</tr>
<tr>
<td></td>
<td>1-3</td>
<td>Any size</td>
</tr>
</tbody>
</table>
| Oncotype DX® Breast Cancer Assay                      | For infiltrating breast cancer to assess the need for adjuvant chemotherapy in a woman when ALL of the following criteria are met:  
  - recently diagnosed stage 1 or stage 2 breast cancer  
  - estrogen receptor positive  
  - HER2-receptor negative  
  - no evidence of distant metastasis  
  - EITHER of the following criteria:  
    - axillary-node status is negative (micrometastasis is no greater than 2.0 millimeters) whether the woman is pre- or post-menopausal  
    - up to three positive axillary nodes in a post-menopausal woman |
| Prosigna® Breast Cancer Assay (PAM50)                 | For breast cancer to assess the need for adjuvant chemotherapy in a woman when ALL of the following criteria are met:  
  - recently diagnosed stage 1 or stage 2 breast cancer  
  - estrogen receptor positive  
  - HER2 receptor negative  
  - postmenopausal  
  - No evidence of distant metastasis  
  - Axillary node status is negative (micrometastasis is no greater than 2.0 mm) |
| VeriStrat® serum proteomic                             | For advanced non-small cell lung cancer (NSCLC) to determine second- |
testing (CPT© Code 81538) line treatment when ALL of the following criteria are met:

- EGFR mutation status is wild-type (i.e., no mutation detected) or unknown
- individual has failed first-line systemic chemotherapy
- test results will be used to decide whether to proceed with erlotinib (Tarceva®) therapy

### Experimental/Investigational/Unproven

The following tumor profile or gene expression tests are considered experimental, investigational or unproven for ANY other indication than noted in the criteria listed above:

- MammaPrint® 70-Gene Breast Cancer Recurrence Assay
- Oncotype DX® Breast Cancer Assay
- Prosigna® Breast Cancer Assay (PAM50)
- VeriStrat serum proteomic testing

Detection of the following is considered experimental, investigational or unproven:

- Circulating whole tumor cells

### Prostate Cancer Screening and Prognostic Tests

### Medically Necessary

The following prostate cancer screening and prognostic genetic tests are considered medically necessary when the associated criteria are met:

<table>
<thead>
<tr>
<th>Test Name</th>
<th>Cancer Type and Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>4K score Test</td>
<td>For prostate cancer when results will impact medical management and the following criterion is met:</td>
</tr>
<tr>
<td>percent free PSA</td>
<td>- PSA &gt;3.0 ng/mL with or without previous benign prostate biopsy</td>
</tr>
<tr>
<td>Prostate Health Index (PHI)</td>
<td></td>
</tr>
<tr>
<td>ConfirmMDx</td>
<td>For prostate cancer when results will impact management and BOTH of the following criteria are met:</td>
</tr>
<tr>
<td>Progensa® PCA3 Assay</td>
<td>- PSA &gt;3.0 ng/mL</td>
</tr>
<tr>
<td></td>
<td>- previous benign prostate biopsy or focal high grade prostatic intraepithelial neoplasia (PIN)</td>
</tr>
</tbody>
</table>

The following prostate cancer screening and prognostic tests are considered experimental, investigational or unproven for ANY other indication:
Medical Coverage Policy: 0520

• 4K score Test
• ConfirmMDx
• percent free PSA
• Prostate Health Index (PHI)
• Progensa® PCA3 Assay

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**Tumor Tissue-Based Molecular Assays for Prostate Cancer**

**Medically Necessary**

The following tumor-based molecular assays for prostate cancer are considered medically necessary when the associated criteria are met:

<table>
<thead>
<tr>
<th>Test Name</th>
<th>Cancer Type and Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decipher® Prostate Cancer Classifier Assay</td>
<td>ANY of the following:</td>
</tr>
<tr>
<td></td>
<td>• PSA persistence after radical prostatectomy (i.e., failure of PSA to fall to undetectable levels after radical prostatectomy)</td>
</tr>
<tr>
<td></td>
<td>• PSA recurrence after radical prostatectomy (i.e., undetectable PSA after radical prostatectomy with a subsequent detectable PSA that increases on two or more determinations)</td>
</tr>
<tr>
<td></td>
<td>• low-risk* or favorable intermediate-risk* prostate cancer</td>
</tr>
<tr>
<td>OncotypeDx Genomic Prostate Score</td>
<td>Post prostate biopsy for low risk* or favorable intermediate-risk* prostate cancer when the individual is a candidate for active surveillance or definitive therapy</td>
</tr>
<tr>
<td>Prolaris® Prostate Cancer Test</td>
<td></td>
</tr>
<tr>
<td>ProMark Proteomic Prognostic Test</td>
<td></td>
</tr>
</tbody>
</table>

*Low-risk: T1-T2a disease AND Gleason score ≤6/grade group 1 AND PSA <10ng/mL
Favorable intermediate-risk: T2b-T2c disease OR Gleason score 3+4=7/grade group 2 OR PSA 10-20 ng/mL AND percentage of positive biopsy cores <50%

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**Hematologic Cancer and Myeloproliferative and Myelodysplastic Disease**

**Medically Necessary**

**Polycythemia Vera (PV)**

JAK2 V617F mutation testing (CPT code 81270) is considered medically necessary for the diagnosis of polycythemia vera (PV) when BOTH of the following criteria are met:
• genetic testing would impact medical management of the individual being tested
• ONE of the following:
  ➢ hemoglobin >16.5 g/dL in men, >16.0 g/dL in women
  ➢ hematocrit >49% in men, >48% in women
  ➢ increased red cell mass (RCM) more than 25% above mean normal predicted value

JAK2 exon 12 mutation testing (CPT code 81403) is considered medically necessary for the diagnosis of PV when ALL of the following criteria are met:
• genetic testing would impact medical management
• Individual meets criteria for JAK2 V617F above
• JAK2 V617F mutation analysis was previously completed and was negative.

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Essential Thrombocythemia

JAK2 V617F mutation testing (CPT code 81270) is considered medically necessary for the diagnosis of essential thrombocythemia or thrombocytosis (ET) when BOTH of the following criteria are met:

• Genetic testing would impact medical management.
• EITHER of the following criteria are met:
  ➢ platelet count ≥ 450 x 10^9/L
  ➢ bone marrow biopsy showing proliferation mainly of the megakaryocyte lineage with increased numbers of enlarged, mature megakaryocytes with hyperlobulated nuclei. No significant increase or left shift in neutrophil granulopoiesis or erythropoiesis and very rarely minor (grade 1) increase in reticulin fibers

MPL and CALR exon 9 mutations (CPT code 81219) common variants (CPT code 81402) are considered medically necessary for the diagnosis of ET when ALL of the following conditions are met:
• Genetic testing would impact medical management.
• Criteria for JAK2 V617F are met.
• JAK2 V617F mutation analysis was previously completed and was negative.

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Primary Myelofibrosis (PMF)

JAK2 V617F mutation testing (CPT code 81270) is considered medically necessary for the diagnosis of primary myelofibrosis (PMF) when BOTH of the following criteria are met:

• Genetic testing would impact medical management.
• Primary myelofibrosis is suspected but not confirmed based on results of conventional testing.

Mutation analysis of MPL common variants (CPT code 81402), MPL exon 10 (CPT code 81403) and CALR exon 9 (CPT code 81219) is considered medically necessary for the diagnosis of PMF when ALL of the following criteria are met:
• Genetic testing would impact medical management.
• Criteria for JAK2 V617F are met.
• JAK2 V617F mutation analysis was previously completed and was negative.
ASXL1, EZH2, TET2, IDH1/IDH2, SRSF2, and SF3B1 testing is considered medically necessary for the diagnosis of primary myelofibrosis (PMF) when ALL of the following criteria are met:

- Genetic testing would impact medical management.
- Megakaryocytic proliferation and atypia, without reticulin fibrosis >grade 1, accompanied by increased age-adjusted bone marrow cellularity, granulocytic proliferation, and often decreased erythropoiesis.
- Above criteria are met.
- JAK2, CALR and MPL mutation analysis was previously completed and was negative.

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**Chronic Myelogenous Leukemia (CML) and Philadelphia Chromosome Positive (Ph+) Acute Lymphoblastic Leukemia (ALL)**

BCR-ABL T315-I mutation testing (81401, 81170) is considered medically necessary in individuals with chronic myelogenous leukemia (CML) or Philadelphia chromosome positive (Ph+) acute lymphoblastic leukemia (ALL) when ANY of the following are met:

- Inadequate initial response to tyrosine kinase inhibitor therapy (i.e., failure to achieve complete hematological response at 3 months, minimal cytogenetic response at 6 months or major cytogenetic response at 12 months).
- Loss of response to tyrosine kinase inhibitor therapy (i.e., hematologic relapse, cytogenetic relapse, loss of major molecular response [MMR]).
- Progression to accelerated or blast phase CML while on tyrosine kinase inhibitor therapy.

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**Occult Neoplasms**

**Medically Necessary**

The following paraneoplastic (onconeural) antibodies are considered 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)

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**Solid Tumor Cancers**

**Experimental/Investigational/Unproven**

Tumor analysis or gene expression profiling for ANY of the following solid tumor types is considered experimental, investigational or unproven (this list may not be all-inclusive):

<table>
<thead>
<tr>
<th>Anal carcinoma</th>
<th>Hodgkin lymphoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal cell carcinoma</td>
<td>Malignant mesothelioma</td>
</tr>
</tbody>
</table>
**Other Tumor Profile Testing**

**Experimental/Investigational/Unproven**

Topographic genotyping for any indication is considered experimental, investigational or unproven.

**Overview**

This Coverage Policy addresses tumor profiling, gene expression assays and molecular diagnostic testing for selected hematology/oncology indications. Somatic mutations are changes in the DNA of cells that are not inherited or passed down by blood relatives. They may occur in any cell of the body except the germ cells (i.e., egg and sperm). These tests are used to identify disease-causing somatic mutations or the biological activity of genes originating in a tumor or hematologic malignancy.

This type of testing can aid in determining the extent or stage of disease, probability of recurrence, appropriate treatment options and how well the disease may respond to treatment in certain clinical scenarios.

**General Background**

For additional information regarding specific genetic tests please refer to the [Genetic Testing-Collateral File](#).

**General Criteria for Somatic Mutation Genetic Testing**

Somatic mutations are changes in the DNA of a cell that may occur in any cell of the body except the germ cells (i.e., egg and sperm). Somatic mutations differ from germline mutations, which are passed down by blood relatives; somatic mutations are not inherited. The genetic tests described in this Coverage Policy are used to identify disease-causing somatic mutations or the biological activity of genes originating in a tumor or hematologic malignancy.

Tumor markers, also known as biomarkers are substances that are produced by certain cells of the body in response to cancer or some noncancerous conditions. Although most tumor markers are made by normal cells as well as by cancer cells, they are produced at much higher levels in cancerous conditions. They can be found in the blood, urine, stool, tumor tissue, or other tissues or bodily fluids of some patients with cancer (National Cancer Institute [NCI], 2017). Tumor marker levels may be useful in determining the extent or stage of disease or recurrence, determining the most effective treatment for a specific disease and how well the disease will respond to treatment.

Published peer-reviewed evidence and professional society/organizational consensus guidelines support testing for certain tumor markers for the screening, staging, diagnosis and management of some types of cancer. However, for other tumor markers there is insufficient evidence to establish clinical utility for informing on improvement of health outcomes.
Gene expression classifier assays identify genetic alterations or biological activity of several genes in the tumor. Such tests may provide a more complete picture of a tumor’s molecular signature and enable a better estimate of the risk of distant recurrence when considered along with other molecular signatures and clinical characteristics (Marrone, 2014). They have been proposed as an adjuvant tool to assist in determining overall survival (OS), recurrence probability, appropriate treatment options and responsiveness to chemotherapy and are not advocated as stand-alone tools. Numerous gene profiling assays are currently marketed for use in the U.S.

To have clinical utility the specific gene or gene biomarker for which testing has been requested, or gene expression classifier assay should be demonstrated in the published, peer-reviewed scientific literature in the form of prospective clinical trial data to improve the diagnosis, management, or clinical outcomes for the individual’s tumor type or disease when the individual is a candidate for a related therapy. The identification of the gene or biomarker should also be required to initiate a related therapy that has been validated by the NCCN as a Category 1, 2A or 2B Level of Evidence and Consensus recommendation as a standard of care. The NCCN recommendations are defined as: Category 1: Based upon high-level evidence there is uniform NCCN consensus that the intervention is appropriate, Category 2A: Based upon lower-level evidence there is uniform NCCN consensus that the intervention is appropriate, Category 2B: Based upon lower-level evidence there is NCCN consensus that the intervention is appropriate and Category 3: Based upon any level of evidence, there is major NCCN disagreement that the intervention is appropriate.

Multigene panels may also provide important information regarding an individual’s tumor type to direct proven therapy or support management changes for hematology-oncology indications. These tests may be clinically useful when sequential testing of individual genes or biomarkers is not feasible because of limited tissue availability, or when urgent treatment decisions are pending and sequential testing would result in a prolonged testing schedule.

There is insufficient evidence in the published, peer-reviewed scientific literature to support molecular testing when the requested gene(s) or biomarker(s) is(are) correlated with a known therapy, but that therapy has not been validated in prospective clinical trials for the specific tumor type or disease site.

**U.S. Food and Drug Administration (FDA)**

FDA approval is not required for the development or marketing of specific gene tumor markers profiling tests, multigene panel tests or gene classifier tests. Many high-complexity tests are laboratory-developed in a Clinical Laboratory Improvement Amendment (CLIA)-certified laboratory. However, a number of 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).

**Tumor Profile/Gene Expression Classifier Testing**

**MammaPrint® 70-Gene Breast Cancer Recurrence Assay**
The MammaPrint® 70-Gene Breast Cancer Recurrence Assay (Agendia, Inc. USA, Irvine, CA) utilizes a deoxyribonucleic acid (DNA) microarray assay to perform 70-gene profiling of breast cancer tissue to assess risk of recurrence. The assay is designed to determine the expression of specific genes in a tissue sample. The result is an expression profile, or “fingerprint”, of the sample. The MammaPrint Index is calculated from fresh, frozen or formalin-fixed paraffin embedded (FFPE) breast cancer tissue and the molecular prognosis profile of the sample is determined (i.e., Low Risk, High Risk) (FDA, 2015).

**U.S. Food and Drug Administration (FDA)**

MammaPrint® 70-Gene Breast Cancer Recurrence Assay (Agendia, Inc. USA, Irvine, CA) received a 510K approval for an individual with Stage I or Stage II lymph node negative breast cancer with a tumor size ≤ 5.0 cm. The test can be performed on fresh, frozen or formalin-fixed paraffin embedded (FFPE) breast cancer tissue samples to assess an individual’s risk for distant metastasis within five years. According to the FDA approval summary, MammaPrint FFPE is not indicated as a standalone test to determine the outcome of disease, nor to
suggest or infer an individual’s likely response to therapy. Results should be taken in the context of other relevant clinicopathological factors and standard practice of medicine (2015).

**Literature Review**

Prospective and retrospective clinical trials in the published, peer-reviewed scientific literature demonstrate the analytical and clinical validity and clinical utility of the MammaPrint assay (Azim, 2013; Bueno-de-Mesquita, 2007; Buyse, 2006; Cardoso, 2016; Drukker, 2013; Gevensleben, 2010) The test has been validated in a woman being considered for adjuvant systemic therapy with Stage I or Stage 2 invasive breast cancer who has estrogen receptor (ER) positive/progesterone receptor (PR) positive, human epidermal growth factor receptor 2 (HER2)-negative disease, and up to three positive lymph nodes, when there is a high clinical risk of recurrence:

<table>
<thead>
<tr>
<th>Tumor Grade</th>
<th>Nodes</th>
<th>Tumor Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well differentiated</td>
<td>None</td>
<td>3.1-5 cm</td>
</tr>
<tr>
<td></td>
<td>1-3</td>
<td>2.1-5 cm</td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>None</td>
<td>2.1-5 cm</td>
</tr>
<tr>
<td></td>
<td>1-3</td>
<td>Any size</td>
</tr>
<tr>
<td>Poorly differentiated or undifferentiated</td>
<td>None</td>
<td>1.1-5 cm</td>
</tr>
<tr>
<td></td>
<td>1-3</td>
<td>Any size</td>
</tr>
</tbody>
</table>

Further, there is consensus support in the form of published guidelines by the American Society of Clinical Oncology ([ASCO], 2017) for the use of MammaPrint to inform decisions on withholding adjuvant systemic chemotherapy due to its ability to identify a good prognosis population with potentially limited chemotherapy benefit.

Cardoso et al. reported five-year outcomes of a subset of patients involved in a randomized phase III trial comparing MammaPrint with Adjuvant! Online in selecting patients with negative, or one-three positive nodes for adjuvant chemotherapy. The Microarray in Node-Negative and 1 to 3 Positive Lymph Node Disease May Avoid Chemotherapy (EORTC 10041/BIG 3-04 [MINDACT]) study enrolled 6600 patients across 112 centers in nine countries and is currently ongoing. Eligible patients were women 18-70 years with histologically confirmed primary invasive breast cancer (stage T1 or T2 or operable T3). In the initial study design, patients had to have lymph-node–negative disease. As of August 2009, the protocol was revised to allow the enrollment of women with up to three positive axillary nodes. Follow-up is 10 years, with 15 year follow-up for those receiving endocrine therapy. MammaPrint and Adjuvant! Online version 8.0 were used to determine genomic and clinical risk, respectively. High clinical risk as defined in the MINDACT study is available in the Supplementary Appendix materials.

Women identified as high clinical/high genomic risk were advised to undergo chemotherapy. Women identified as low clinical/low genomic risk were advised not to undergo chemotherapy. Patients with discordant results (i.e., high clinical risk/low genomic risk (n=1550); low clinical risk/high genomic risk (n=592)) were randomly assigned to the chemotherapy group or the no-chemotherapy group on the basis of either the clinical result or the genomic result. The primary end point was survival without distant metastasis (i.e., event-free rate) at five years; secondary end points were the proportion of patients who received chemotherapy according to clinical/genomic risk status and disease-free survival.

Five-year findings published in August 2016 suggest that women with high clinical risk, but low genomic risk based on the MammaPrint assay may avoid adjuvant chemotherapy. Among patients in the intention-to-treat population at high clinical risk and low genomic risk at enrollment, those who underwent randomization on the basis of clinical risk and therefore received chemotherapy had a five-year rate of survival without distant metastasis of 95.9%. Those who underwent randomization on the basis of genomic risk and received no chemotherapy had a rate of 94.4%, 1.5 percentage points lower than the rate among those who received chemotherapy (p = 0.27). Among patients at low clinical risk and high genomic risk, those who underwent randomization on the basis of genomic risk and therefore received chemotherapy had a five-year rate of survival.
without distant metastasis of 95.8% as compared with a rate of 95.0% among those who underwent randomization on the basis of clinical risk and received no chemotherapy (p=.66). No advantage of directing therapy on the basis of genomic risk among patients at low clinical risk but high genomic risk was noted. In addition, among patients in the discordant risk groups, there were no significant differences between the chemotherapy and no-chemotherapy groups with respect to disease-free survival and overall survival. The authors note that approximately 46% of women with breast cancer who are at high clinical risk might not require chemotherapy. Data suggest MammaPrint was significantly associated with survival without distant metastasis after adjustment for chemotherapy use, clinical risk, and patient and tumor characteristics in a multivariate analysis (p<0.001).

The Microarray Prognostics in Breast Cancer (RASTER) trial prospectively evaluated the impact of MammaPrint in assisting with adjuvant treatment decisions in 427 women with primary breast cancer. The prognostic results from MammaPrint were shown to have an impact on physicians’ decisions for adjuvant treatment in 20% of patients (Drukker, 2013).

The IMProving care And Knowledge through Translational research (IMPAKT) 2012 Working GROUP Consensus published a critical evaluation of the analytical validity, clinical validity and clinical utility based on the principles of the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) criteria. IMPAKT found convincing evidence of analytical and clinical validity; however, robust evidence of clinical utility was not shown as the studies did not show clear evidence in improvement of clinical outcome utilizing the MammaPrint ® assay (Azim et al., 2013).

Professional Societies/Organizations
For a summary of professional society recommendations/guidelines regarding MammaPrint 70-Gene Breast Cancer Recurrence Assay please click here.

Oncotype DX® Breast Cancer Assay: According to the manufacturer (Genomic Health, Inc., Redwood City, CA), this test is recommended for use after the original breast cancer surgery and is proposed for newly diagnosed patients with node-negative or node-positive, ER-positive, HER2-negative invasive breast cancer (Genomic Health, 2004-2015). The purpose of the Oncotype DX Breast Cancer Assay is to quantify the likelihood of distant recurrence (i.e., within 10 years) in a woman with breast cancer, and is used as one factor in determining whether or not a patient is a candidate for chemotherapy. This assay is not proposed for or used as a test to monitor the response of a specific chemotherapy drug.

Using tumor tissue, ribonucleic acid (RNA) is extracted, purified and analyzed for expression of a panel of 21 genes using quantitative reverse transcription polymerase chain reaction (RT-PCR) on formalin-fixed, paraffin-embedded (FFPE) tumor tissue. A Recurrence Score™ (RS) is calculated from the gene expression results using a proprietary Oncotype DX algorithm. The RS is based on a scale of 0–100. A score of less than 18 is considered low-risk; 18-31 is intermediate-risk; and a score over 31 is designated as high-risk. Each RS correlates with a specific likelihood of distant recurrence at 10 years. This test is recommended by the American Society of Clinical Oncology (ASCO) (2007) and NCCN (2015) for use in a select population of women with breast cancer.

The published peer-reviewed evidence in the form of retrospective analyses and observational studies support the accuracy and clinical utility of Oncotype DX in its ability to predict the benefits of chemotherapy in women with localized stage 1 or stage 2 breast cancer who are ER-positive HER2-receptor negative and are axillary node negative in pre- and postmenopausal women, with no evidence of metastasis. Although data are not robust, the role of Oncotype DX for use by women with axillary node positive (i.e., 1-3 nodes) early breast cancer is supported by the published NCCN consensus guideline for breast cancer.

Data regarding Oncotype DX for other indications, including men with breast cancer, ductal cancer in situ and the value of repeat assays after the initial assessment are insufficient to establish clinical utility. Furthermore, professional society/organization consensus support by way of published guidelines or practice statements are lacking for these patient subsets.

US Food and Drug Administration (FDA)
Oncotype DX has not received U.S. Food and Drug Administration (FDA) approval. The assay is performed in the licensed Genomic Health laboratory where the assay was developed.

**Literature Review**

Supporting data on the clinical utility Oncotype DX in men, use in ductal carcinoma in situ (DCIS) and the value of repeat assays after the initial assessment are lacking. The body of published peer-reviewed evidence establishing the clinical usefulness of this test for a woman with early breast cancer includes several prospective studies and retrospective analyses of archived tumor samples from previous randomized controlled clinical trials. High-level evidence, in the form of randomized clinical trial data are lacking. However, the NCCN Guideline for Breast Cancer (2015) considers the test as a Category 2A level of evidence and consensus recommendation to guide the use of chemotherapy in node negative and node positive women with early breast cancer who are estrogen-receptor positive HER2-receptor negative and have no evidence of distant metastasis.

The published peer-reviewed literature supports the accuracy and clinical utility of Oncotype DX in its ability to predict the benefits of chemotherapy in a woman with localized stage 1 or stage 2 breast cancer who is ER-positive and HER2-receptor negative with no evidence of metastasis. The woman should be pre-or postmenopausal and have axillary-node-negative disease, which includes micrometastasis no greater than 2.0 millimeters, or postmenopausal for a woman with axillary node positive disease (i.e., up to three nodes).

**Node Negative Disease**

According to the published peer-reviewed scientific literature, women with node-negative disease with a low recurrence score (RS) had a 10-year distant recurrence-free survival rate of 93.2%; 85.7% for patients with an intermediate RS, and 69.5% for patients with a high RS (Tang, et al., 2011; Lo, et al., 2010; Mamounas, et al., 2010; Toi, et al., 2010; Chang, et al., 2008; Habel, et al., 2006; Paik, et al., Aug 2006; Cobleigh, et al., 2005; Gianni, et al., 2005; Paid, et al., 2004). However, in a critical evaluation by the IMPAKT 2012 Working GROUP Consensus, robust evidence of clinical utility was not shown (Azim, 2013).

Carlson et al. conducted a recent systematic review and meta-analysis of 23 studies to synthesize the results and provide insights about the utility Oncotype DX. Oncotype DX changed the clinical-pathological adjuvant chemotherapy recommendation in 33.4% of patients (eight studies, 1,437 patients). High recurrence score (RS) patients were significantly more likely to follow the treatment suggested by Oncotype DX compared with patients with a low RS (RR: 1.07 (1.01–1.14). The randomized prospective TAiLORx ongoing clinical trial is ongoing to assay the treatment results and clinical utility of treatment decisions made by Oncotype DX risk stratification (Sparano et al 2008).

Based on a systematic review of the literature, a BlueCross BlueShield technology assessment (2014) concluded that retrospective analyses of tumor samples indicate strong, independent associations between Oncotype DX RS results and distant disease recurrence or death in women with lymph node-negative breast cancer. There are several limitations to the available evidence: 1) Among those willing to be guided by the test result, it is unknown what proportion of conventionally estimated intermediate- to high-risk patients will have sufficiently low RS values to change their decision regarding chemotherapy. 2) How the recurrence risk level below which women might choose to forgo chemotherapy is affected by patient preferences, needs, and values is unknown. 3) Women reclassified by RS result as intermediate or high risk from conventionally estimated low risk have a much wider range of recurrence risk estimates; women with very high RS values are likely to benefit from accepting chemotherapy; but for the intermediate-risk group, benefits are uncertain. 4) Because RS is a continuous function with respect to recurrence rates, risk category cutoff values selected by the test developer may not be optimal. Although some patient evaluation and treatment regimens in published studies differ from current practices, findings with respect to the utility of gene expression profiling using Oncotype DX®. Use of aromatase inhibitor–based hormonal therapy instead of tamoxifen therapy does not change and would likely reduce recurrence rates for all RS risk groups.

**Node Positive Disease**

The test has also been proposed for use with postmenopausal women with hormone-receptor positive node-positive (1–3 nodes) breast cancer in the published, peer-reviewed scientific literature (Eirman, 2012; Solin, 2012; Albain, 2010; Dowsett, 2010). Although data are not robust regarding the test’s prognostic ability for this
As previously noted, the clinical usefulness has been validated by the NCCN as a category 2A level of evidence and consensus recommendation.

Chung and Christianson (2014) published results of a systematic review regarding the usefulness of predictive and prognostic biomarkers with therapeutic targets in breast, colorectal, and non-small cell lung cancers. Systematic reviews and meta-analyses of published predictive (associated with treatment response and/or efficacy) and prognostic (associated with disease outcome) biomarkers of known therapeutic targets in colorectal, breast, and non-small cell lung cancers were evaluated. Based on low level evidence the authors found that Oncotype DX (21-gene RT-PCR expression assay) is an emerging prognostic marker for early-stage ER-positive, node-negative patients. Individuals with a high recurrence score (31 or above): patients have poor prognosis and may need adjuvant chemotherapy in combination with hormonal therapy. Low recurrence score (18 or lower): patients have favorable prognosis and may not need chemotherapy. Intermediate recurrence score (19–30): prognostic value of Oncotype DX not known, awaiting prospective trial results.

Eirmann et al. (2012) reported results of a prospective study to evaluate the impact of Oncotype DX on adjuvant decision-making in a cohort of consecutive patients with ER+, HER2-negative early breast cancer (EBC). Secondary study objectives assessed the impact of the RS on patients’ decisional conflict, on physicians’ confidence in their treatment recommendations, to evaluate the rate of therapy actually administered in relation to recommended therapies, and to assess the pharmaco-economic impact of RS-guided adjuvant decision-making for German clinical practice. Eligible female patients presented with operable EBC, ER positive, HER2 negative by IHC or FISH, tumor size of ≥1 cm (i.e., T1, 2, 3 excluding those with dermal involvement) or <1 cm if at least one histological unfavorable characteristic (i.e., high histological grade, angiolymphatic invasion and p-53 positive), node-negative or histologically verified lymph node metastases in up to three lymph nodes. Further inclusion criteria were age ≥18 years, good performance status (ECOG 0–1, Karnofsky Index ≥70) and no contraindication for receiving systemic chemoendocrine therapy.

There were 366 assessable patients; 244 were node-negative (67%), and 122 node-positive (i.e., 1-3 nodes, 33%). After the recurrence score (RS) was known the initial treatment recommendation was revised in 33% of all assessable patients (30% in node-negative (N0) and 39% in node-positive (N+) subgroups). Overall, physicians’ confidence increased in 45% of N0 patients and 46% of N+ cases (P < 0.001). Completed questionnaires were available for 325 patients. The difference from the pre- to post-test decision conflict score (DCS) was statistically significant for all patients (6%, P = 0.028) and for patients with low RS values (11%, P = 0.003). Treatment administered differed from the post-RS recommendation in 45 cases (9% of patients with N0, and 20% with N+ disease). There was an overall 19% net reduction in adjuvant chemotherapy usage when the actual number of chemotherapies given was compared with the initial treatment recommendation regardless of the nature of the pre-test recommendation. Data suggest that there is significant influence relative to knowledge of the RS and resulting treatment recommendations.

Hornsberger et al. (2012) conducted a systematic review of the literature assessing clinical validity/utility, change in clinical practice, and economic implications of the following early stage breast cancer stratifiers: 21-gene recurrence score (Oncotype DX), 70-gene signature (MammaPrint), 5-gene expression index (Molecular Grade Index, bioTheranostics, Dan Diego, CA), 5-antibody immunohistochemistry (IHC) panel (Mammostrat, Clarient, Alisa Viejo, CA), and the 14-gene signature (BreastOncPx, US Labs, Irvine, CA). Clinical validity was defined as the ability of the assay to predict the clinical endpoint(s) of interest. Clinical utility was defined as the balance of associated benefits and risks if the assay is introduced into clinical practice. The primary objective was to systematically grade the Level-of-Evidence (LOE) of the eligible studies. The secondary objective was to document studies that provide evidence on changes in practice patterns and health economic implications of the stratifiers. Studies were graded as Category A, representing prospective, randomized clinical trial designs, Category B: prospective studies using archived tissue samples, Category C: prospective, observational registry studies in which treatment and follow-up are not dictated, and Category D representing retrospective/observational studies. The authors note “Category B was included in this framework because a positive result from this type of study is less likely to be a ‘play of chance’ than a similar result from a Category C, prospective, observational registry.”

Fifty-six articles were included in the review including the 21-gene recurrence score (n = 31, 14 related to prognosis or prediction), 70-gene signature (n = 14, 11 related to prognosis or prediction), Adjuvant! Online (n =
12, 5 related to prognosis or prediction), 5-antibody immunohistochemistry panel (n = 3, all related to prognosis or prediction), 5-gene expression index (n = 1, related to prognosis or prediction), and 14-gene signature (n = 1, related to prognosis or prediction). According to the review, there was Level 1 evidence that the 21-gene recurrence score estimated distant recurrence risk (DRR), overall survival (OS), and response to adjuvant chemotherapy, and Level II evidence estimating local recurrence risk. There was Level II evidence that the 5-antibody immunohistochemistry panel and 70-gene signature estimated DRR and OS. There was Level II evidence that Adjuvant! Online, a software model that predicts the benefit of adjuvant therapy, estimated DRR, OS, and chemotherapy response. The five-gene expression index satisfied Level III evidence for predicting DDR and the 14-gene signature satisfied Level III evidence for predicting DRR and OS. No recommendations were made regarding the application of these assays into routine clinical practice.

Solin et al. (2012) retrospectively evaluated 388 tumor samples to assess the significance of biologic subtype and 21-gene recurrence score relative to local recurrence and local–regional recurrence after breast conservation treatment with radiation. Specimens were taken from the Eastern Cooperative Oncology Group E2197 randomized controlled trial (RCT) that compared two adjuvant systemic chemotherapy regimens. Patients had operable breast cancer, 0–3 positive lymph nodes and tumor size > 1.0 centimeters. The subset for this study was patients with known 21-gene recurrence scores (RS) and treated with surgery, systemic chemotherapy, and definitive radiation treatment. Follow-up ranged from 3.7-11.6 years (median, 9.7 years). Ninety patients had one positive node, 41 patients had two positive nodes and 16 patients had three positive nodes.

Neither biologic subtype nor the RS was associated with local recurrence or local–regional recurrence on univariate analyses (p>0.12, each). The 10-year local recurrence rates were 3.1%, 2.9% and 7.6 % for low, intermediate, and high RS, respectively (p=0.24). Analysis of the RS as a continuous variable, restricted to HR-positive tumors yielded a borderline statistically significant hazard ratio for local recurrence (p=0.07) and a statistically significant hazard ratio for local–regional recurrence (p<0.03). No significant differences were seen when the RS was combined with patient age (p=0.09), HR positive tumors (p=0.02), or HER2-negative tumors (p=0.02). Analysis of RS combined with HR positive, HER2-negative tumors showed a significant result for local and local–regional recurrence when adjusted for age (p≤0.03). Multivariate analyses for local recurrence and local–regional recurrence using the variables of chemotherapy arm, patient age, HR status, pathologic axillary lymph node status, histologic grade, pathologic T stage, biologic subtype, and RS identified no statistically significant variables (p≥0.26, each). Subgroups identified with 10-year recurrence > 10% were 1) HR-positive tumors with a high RS; 2) HR positive, HER2-negative with a high RS; and 3) patients age ≤ 39 years. However, the authors cautioned that the results in these subgroups were based on a small number of patients with wide 95% confidence intervals. In this study, the rates of local recurrence and local–regional recurrence were higher with increasing RS but were not statistical significant. Results related to number of positive nodes were not reported. Author-noted limitations include: patients treated in this study predated the era of adjuvant trastuzumab for HER2-positive tumors (15% in this study), the biologic subtype was approximated, and the study represented a subset of the overall population in the original E2197 study. Additional study limitations include retrospective design and limited data regarding impact on patient management.

Dvorak et al. (2012) compared the HER2 status documented on Oncotype DX reports (n=194) to the HER2 results conducted by fluorescence in situ hybridization (FISH) in the same patients to determine the frequency of discrepancy between HER2 status performed by these two tests and to characterize the clinicopathologic features of discrepant cases. Overall agreement was 96%, negative agreement was 100%, and percent positive agreement was 50%. Three of eight (38%) discrepant cases showed heterogeneous amplification by FISH. Seven of eight (88%) cases had < 50% invasive tumor in the Oncotype DX tissue block. In three of eight discrepant cases, the blocks used for FISH and those sent for Oncotype DX testing were different. Seven discrepant cases had < 50% of the surface area involved by invasive cancer.

Dowsett et al. (2010) evaluated 1231 tissue samples from a previously reported randomized controlled trial to determine the prognostic value of Oncotype DX for distant recurrence in hormone receptor-positive postmenopausal women with localized node-negative (NO) (n=872) or node-positive (N+) (n=306) breast cancer patients who were treated with either tamoxifen (n=609) or anastrozole (n=622). The samples were obtained from the tamoxifen and anastrozole arms of the Arimidex, Tamoxifen, Alone or in Combination (ATAC) Trial (n=4160) which evaluated the safety and efficacy of five years of anastrozole, tamoxifen, or both in
postmenopausal women. Sixty-three patients had ≥ 4+ nodes and 243 patients had 1–3 positive nodes. Node status was unknown in the remaining patients. In the N+ group, 152 women received tamoxifen and 154 received anastrozole. The median follow-up was 8.5 years. Tumor size and the Oncotype DX Recurrence Score (RS) were each separately statistically significant (p<0.001 each) in predicting time to distant recurrence (TTDR) in node-negative (N0) patients. The RS was also predictive of TTDR in node positive (N+) patients (p=0.002) in multivariate analyses. The number of positive nodes (p<0.001) and tumor size (p=0.006) were also statistically significant variables in multivariate analyses. The rates of distant recurrence (DR) at 9 years in the N0 patients were 4% in the RS < 18 group, 12% in the RS 18–30 group, and 25% in the RS ≥ 31 groups and in the N+ groups, 17%, 49%, and 64% respectively. The overall survival (OS) rates at 9 years in the N0 patients were 88% in the RS < 18 group, 84% in the RS 18–30 group, and 73% in the RS ≥ 31 groups and in the N+ group, 74%, 69%, and 54%, respectively. Seventy-two N0 patients, 74 N+ patients and six node unknown patients experienced distant recurrence (DR). The DR rate increased linearly with an increase in RS. The risk was higher for N+ and for patients with ≥ four positive nodes. There was no significant difference in the RS and risk of DR by treatment or by N0 or N+. The prognostic information from the RS was independent of the prognostic information of Adjuvant! Online. Although the study did not directly evaluate the value of RS in predicting the benefit of chemotherapy, the data indicated that the RS adds to the information provided by node status, patient age, tumor size and tumor grade and proposed that RS may be added to treatment decisions regarding chemotherapy.

In a retrospective review of a randomized controlled trial (i.e., Southwest Oncology Group (SWOG)-8814, INT-0100 study), Albain et al. (2010) investigated the ability of the Oncotype DX recurrence score to determine the prognosis of node-positive (N+) women (n=227) treated with tamoxifen alone and those who might not benefit from anthracycline-based chemotherapy. The study included postmenopausal women with axillary node-positive (1–3 vs. ≥ 4 nodes) breast cancer and either ER-positive or PR-positive tumors. Patients were randomized to treatment with tamoxifen alone for five years (n=148; 94 with 1-3 positive nodes) or to treatment with cyclophosphamide, doxorubicin, and fluorouracil followed by tamoxifen (CAF-T) (n=219; 133 with 1–3 positive nodes). Patients in this subset had a slightly lower number of positive nodes and smaller tumor size (< 2 cm [n=46], 2–5 cm [n=94], > 5 cm [8]) compared to the parent trial, and 11.7% were HER2 positive. The samples were analyzed using the RT-PCR Oncotype DX assay. The recurrence score was significantly prognostic in the tamoxifen-only group (p=0.006). No benefit was identified in CAF-T patients with a recurrence score < 18 (p=0.97). There was however, an improvement in disease-free survival for those with a high recurrence score ≥ 31 (p=0.033). The recurrence score by treatment interaction was significant only in the first five years (p=0.029), but the cumulative benefit was present at ten years. The results of the study suggested that patients with a low recurrence score and 1–3 involved axillary lymph nodes did not benefit from anthracycline-based chemotherapy, but those with a higher recurrence score had major benefit, independent of the number of positive nodes. Although the study provided further data on the prognostic value of Oncotype DX for post-menopausal women with ER-positive, 1–3 node-positive breast cancer treated with adjuvant tamoxifen, the authors noted the following limitations: it is unknown if the results of the study can be applied to premenopausal women.

Using tumor samples (n=465) from a previous randomized controlled trial, Goldstein et al. (2008) conducted a retrospective clinical trial to evaluate the prognostic utility of Oncotype DX in either node-negative or node-positive, hormone receptor-positive breast cancer patients treated with doxorubicin-containing chemotherapy and to determine if Oncotype DX could more reliably predict outcomes at five years than standard clinicopathologic features. The study included pre- (41.4%) and postmenopausal (58.6%) women, HER2 positive (21.9%) and HER2 negative (44.0%) (HER2 status was unknown in 34.1% of women). Axillary node status included 56.5% negative nodes, 24.0% one positive node, 13.5% two positive nodes, and 6.1% three positive nodes. Tumor sizes included ≤ 2.0 cm (52.9%), 2.1–5.0 cm (42.5%), and > 5.0 cm (3.6%). All patients received chemotherapy. The median follow-up was six years. The authors used an integrator that was modeled after Adjuvant! but adjusted for five-year outcomes, evaluated the concordance between RS prediction and the integrator, compared the RS predictive accuracy with the integrator, and evaluated if RS provided additional information regarding relative risk of recurrence. The results indicated that the RS was significantly predictive of recurrence in patients with and without positive nodes (p=0.001) compared to clinicopathologic features and a clinical algorithm. Approximately 3.3% of patients with a recurrence score < 18 with 0–1 positive nodes experienced recurrence within five years compared to 7.9% with 2–3 positive nodes. Limitations of the study include the retrospective study design and the heterogeneous patient population.
Cobleigh et al. (2005) conducted a retrospective validation study which involved the analysis of paraffin block specimens from women (n=86) with invasive breast cancer with greater than 10 positive nodes and no evidence of metastases. Quantitative gene expression was determined by a multianalyte Taqman RT-PCR (i.e. Oncotype DX). Seven samples were inadequate and were not used. Diagnosis included infiltrating ductal carcinoma (n=68) and infiltrating lobular carcinoma (n=9). One patient had both types of cancer. Mean tumor size was 4.4 ± 3.3 centimeters and the number of positive nodes ranged from 10–40 (median 15). A total of 54% of the women received adjuvant tamoxifen and 80% received adjuvant chemotherapy. Median follow-up was 15.1 years and median time to distant recurrent or death was 2.6 years. Only the number of nodes involved was significantly associated with distant recurrence-free survival (DRFS) (p<0.05) with a 4% increase in risk for each additional involved node. HER2 expression was significantly correlated with DRFS (p<0.001). HER2/immunohistochemistry remained significant (p<0.05) with regard to clinical and pathologic measurements. Clinical and pathologic variables (e.g., age, tumor size, number of involved nodes, systemic treatment) had only “modest correlation” with recurrence. The two pathologists agreed on 57.6% of the patients’ tumor grades. Analysis of the 21 genes in the recurrence score (RS) showed 14 of the 16 cancer-related genes in the RS correlated with breast cancer recurrence (p<0.05 for nine genes and p<0.10 for 14 genes). A total of 11 patients (14%) had a RS < 18 and a 10-year distant recurrence rate of 29%; 19 patients (24%) had a RS between 18 and 31 and a 10-year distant recurrence rate of 72%; and 48 patients (62%) had a RS of ≥ 31 and a 10-year distant recurrence rate of 80%. Overall, concordance between Oncotype DX and immunohistochemistry for ER, PR and HER2 was high, but poor for Ki-67. The authors noted that the sample size in this study for node-positive women was insufficient to “address the relative performance of the Recurrence Score and standard measures, such as patient age, tumor size, and tumor grade” and that although these data were used to develop Oncotype DX, the RS cannot be considered validated in this node-positive subpopulation.

Professional Societies/Organizations
For a summary of professional society recommendations/guidelines regarding Oncotype DX Breast Cancer Assay please click here.

Prosigna® Breast Cancer Prognostic Gene Signature Assay: Prosigna® (NanoString Technologies, Seattle, WA) is an in vitro diagnostic assay which is performed on the NanoString nCounter® Dx Analysis System using formalin-fixed paraffin embedded (FFPE) breast tumor tissue previously diagnosed as invasive breast carcinoma. It is designed to identify intrinsic breast cancer subtypes (i.e., luminal A/B, HER2 enriched, basal like) and generate a Risk of Recurrence (ROR) score, expressed as a numerical value (0-100 scale) which correlates with the probability of distant recurrence within 10 years. The Prosigna Risk of Recurrence (ROR) score is generated by Prediction Analysis of Microarray (PAM50) proprietary algorithm (NanoString Technologies, 2014-2016)

U.S. Food and Drug Administration (FDA)
Prosigna received FDA 501K approval in September, 2013. According to the FDA, the Prosigna Breast Cancer Prognostic Gene Signature Assay is indicated in female breast cancer patients who have undergone surgery in conjunction with locoregional treatment consistent with standard of care, either as:

- A prognostic indicator for distant recurrence-free survival at 10 years in postmenopausal women with Hormone Receptor-Positive (HR+), lymph node-negative, Stage I or 11 breast cancer to be treated with adjuvant endocrine therapy alone, when used in conjunction with other clinicopathological factors.
- A prognostic indicator for distant recurrence-free survival at 10 years in postmenopausal women with Hormone Receptor-Positive (HR+), lymph node-positive (1-3 positive nodes), Stage 11 breast cancer to be treated with adjuvant endocrine therapy alone, when used in conjunction with other clinicopathological factors.

Prosigna is not intended for diagnosis, to predict or detect response to therapy, or to help select the optimal therapy for patients. The device is not intended for patients with four or more positive nodes

Literature Review
High-level evidence in the form of randomized controlled clinical trial data is lacking. The majority of studies reported in the peer-reviewed scientific literature are retrospective and all used archived FFPE breast tumor
tissue to determine outcomes (Gnant, 2014; Dowsett, 2013; Filibits, 2013; Sestak, 2013; Nielsen, 2010). Several studies utilized archived tumor samples of patients previously enrolled in randomized controlled clinical trials (Gnant, 2014; Dowsett, 2013; Filibits, 2013). Statistically significant correlation with 10-year distant recurrence was noted in all studies. Weurstlein et al. (2013) demonstrated overall concordance at the individual tumor level between subtype classification by treating physician assessment and by Prosigna results (71.7%) and a change in the adjuvant therapy recommendation occurred in 36 (18.2%) patients; 22 (11.1%) patients switched from no chemotherapy to chemotherapy. Increased confidence in their prognostic assessment and treatment recommendation was noted in 87.9% of physicians.

Although data are not robust, in the form of randomized controlled clinical trial data, there are data to support the use of Prosigna to assess the need for adjuvant chemotherapy in a postmenopausal woman with recently diagnosed stage 1 or 2 breast cancer, who is estrogen receptor positive, HER2 receptor negative and who is axillary node negative with no evidence of distant metastasis. The role of Prosigna for women with node positive disease has not yet been established

**Professional Society/Organizations**

For a summary of professional society recommendations/guidelines regarding Prosigna Breast Cancer Prognostic Gene Signature Assay please click [here](#).

**VeriStrat® Serum Proteomic Testing (CPT® Code 84999):** VeriStrat® (Biodesix, Boulder, CO) is not an EGFR mutation test. It is a serum protein analysis for advanced non-small cell lung cancer (NSCLC) and has been proposed as a means to identify individuals who should receive treatment with erlotinib (Tarceva®, Genentech, San Francisco, CA), an epidermal growth factor inhibitor (EGFRI). According to the Biodesix website, the test stratifies individuals who are likely to have good or poor outcomes with EGFRI treatment (2015). The analysis utilizes matrix-assisted laser desorption/ionization mass spectrometry to analyze serum for eight discriminating features. The test has an established prediction algorithm which was validated in two separate populations. Classifications based on spectra acquired at the two institutions had a concordance of 97.1%. (Taguchi, 2007).

The clinical utility of VeriStrat has been validated in both retrospective and prospective trials as a means to identify an individual who should receive treatment with erlotinib (Tarceva®, Genentech, San Francisco, CA), an epidermal growth factor inhibitor (EGFRI).

**Literature Review**

The clinical utility of VeriStrat is supported by prospective and retrospective clinical trial evidence in the published, peer-review scientific literature. The utility of VeriStrat as compared to standard KRAS and EGFR mutation analysis was performed on 102 samples by Amann et al (2010). VeriStrat classification identified 64 of 88 (73%) as predicted to have “good” and 24 of 88 (27%) predicted to have “poor” outcomes. Statistically significant correlation to VeriStrat status and clinical survival outcome was demonstrated (p<0.001).

Cost utility analysis of applying VeriStrat to guide treatment for NSCLC patients was compared to all patients receiving treatment with EGFRI, all patients receiving chemotherapy; and treatment determined by performance status. Patients where treatment was guided by VeriStrat showed the second best survival outcome (9.6 months) when compared to chemotherapy only (10.1 months); Performance status indicated (9.2 months) and EGFRI only (8.2 months) (Nelson, 2013).

Carbone et al. (2012) reported results of a retrospective analysis of 436 patient samples with NSCLC that were tested in patients treated with erlotinib and those on placebo. VeriStrat status was prognostic for overall survival and progression free survival, independent of clinical features (p=0.002); however, it was not predictive of differential survival from erlotinib over placebo (p=0.48). Similar results were found for progression-free survival. Data suggest a predictive effect of VeriStrat for response to erlotinib.

Subsequent studies have also sought to determine the predictive value of VeriStrat testing. Sun et al (2014) conducted a meta-analysis of current relevant publications. Eleven cohorts involving 706 patients collected from seven studies were subjected to final analysis. The statistical analysis of these articles found that the test’s
“good” status predicted better clinical outcome for overall and progression free survival (p<0.001 for both overall and progression-free survival).

A recent blinded randomized clinical trial by Gregorc et al. (2014) analyzed data collected through PROSE, a biomarker-stratified randomized phase III trial of 285 patients with stage IIIB or IV NSCLC from 14 centers across Italy. The proteomic test classification was masked for patients and investigators who gave treatments, and treatment allocation was masked for investigators who generated the proteomic classification. The primary endpoint was overall survival and the primary hypothesis was the existence of a significant interaction between the serum protein test classification and treatment. A significant interaction between treatment and proteomic classification was noted. Patients who were classified as “poor” in regards to their serum protein test status (30% of participants) were more likely to have better outcomes on chemotherapy than on erlotinib (p=0.022). The data suggests that this subset of patients should not receive erlotinib. This supports the use of a multivariate serum protein test in predicting overall survival for erlotinib versus chemotherapy in second-line therapy. However, there was no difference in treatment observed for patients with the classification of “good” (p=0.714). Although the study demonstrates which patients will not benefit from treatment with erlotinib (“poor” status), additional studies are needed to determine the best treatment option for patients with “good” status.

Circulating Whole Tumor Cell Testing
Circulating whole tumor cells (CTCs) have been found in the peripheral blood circulation of individuals with various forms of metastatic cancer. These are whole cells that have been shed by the tumor. The detection and testing of these tumor cells has been proposed as a method to stratify risk, monitor progression and monitor response to treatment.

The use of circulating whole tumor cell testing has not been proven to impact meaningful health outcomes in an individual with cancer. There are no conclusive data in the published in the peer-reviewed medical literature to date to indicate that knowledge of this prognostic factor can be used to alter the therapy that is offered to patients and improve outcomes. While this testing may have potential for use in patient monitoring, there is currently insufficient evidence to determine the effectiveness of this technology as a marker of disease progression. Additionally, no head-to-head trials have demonstrated that this technology is equal to or better than any existing tumor markers in its efficacy and clinical utility.

There is insufficient evidence to establish the clinical significance of circulating whole tumor cells and how identification can improve health outcomes for an individual with cancer. While pilot studies suggest that the identification of these whole tumor cells may have a role in risk stratification and monitoring responses to treatment, larger longitudinal studies with standard techniques in clearly-defined populations of patients are needed to establish the role of such testing. The role of this testing in patient management is not yet known.

Literature Review
Breast Cancer
Smerage et al. (2014) reported on a randomized trial of patients with persistent increase in CTCs that were tested to determine whether changing chemotherapy after one cycle of first-line chemotherapy would improve the primary outcome of overall survival (OS). Five hundred ninety-five female patients were included with histologically confirmed breast cancer and clinical and/or radiographic evidence of metastatic disease. Patients who underwent chemotherapy had evaluation for CTCs at baseline and then after one cycle. Women whose CTCs remained elevated after the first cycle of therapy (arm C) (n=123) were randomly assigned to either maintain the initial treatment plan (n=64) or to change of chemotherapy (n=59). Changing to an alternate regimen had no difference in OS compared with continuation of the initial regimen (median 12.5 versus 10.7 months, respectively, P= .98). The CTCs did appear to have prognostic value: the median OS for arms A, B, and C were 35 months, 23 months, and 13 months, respectively). While it appears that there is prognostic value of CTCs, the role in clinical management is has not been demonstrated.

Zhang et al. (2012) reported on a meta-analysis of published literature on the prognostic relevance of CTC, including patients with early and advanced disease. Forty-nine eligible studies with 6,825 patients were identified. The main outcomes analyzed were overall survival (OS) and disease-free survival (DFS) in early-stage breast cancer patients, as well as progression-free survival (PFS) and OS in metastatic breast cancer patients. Pooled hazard ratio (HR) and 95% confidence intervals (CIs) were calculated using the random and the
fixed-effects models. The presence of CTC was significantly associated with shorter survival in the total population. The prognostic value of CTC was significant in both early (DFS: HR, 2.86; 95% CI, 2.19–3.75; OS: HR, 2.78; 95% CI, 2.22–3.48) and metastatic breast cancer (PFS: HR, 1.78; 95% CI, 1.52–2.09; OS: HR, 2.33; 95% CI, 2.09–2.60). Subgroup analyses showed that our results were stable irrespective of the CTC detection method and time point of blood withdrawal. The authors conclude that the meta-analysis indicates that the detection of CTC is a stable prognosticator in patients with early-stage and metastatic breast cancer; however further studies are required to explore the clinical utility of CTC in breast cancer.

A prospective observational study that compared serum marker levels with CTC in 267 metastatic breast cancer patients (Bidard, et al., 2012). The secondary pre-planned endpoint a study that previously reported on CTC as prognostic factor (Pierga, et al., 2011), compared prospectively the positivity rates and the value of CTC (CellSearch), of serum tumor markers (carcinoembryonic antigen (CEA), cancer antigen 15.3 (CA 15-3), CYFRA 21-1), and of serum non-tumor markers (lactate deshydrogenase (LDH), alkaline phosphatase (ALP)) at baseline and under treatment for PFS prediction, independently from the other known prognostic factors, using univariate analyses and concordance indexes. The study reported that a total of 90% of the patients had at least one elevated blood marker. The blood markers were correlated with poor performance status, high number of metastatic sites and with each other. CYFRA 21-1, a marker usually used in lung cancer, was elevated in 65% of patients. A total of 86% of patients had either CA 15-3 and/or CYFRA 21-1 elevated at baseline. Each serum marker was associated, when elevated at baseline, with a significantly shorter PFS. Serum marker changes during treatment, assessed either between baseline and the third week or between baseline and weeks six-nine, were significantly associated with PFS, as reported for CTC. Concordance indexes comparison showed no clear superiority of any of the serum marker or CTC for PFS prediction. The authors concluded that for the purpose of PFS prediction by measuring blood marker changes during treatment, currently available blood-derived markers (CTC and serum markers) had globally similar performances. There was no clear superiority found of CTC over the other serum markers.

Liu et al. (2009) conducted on a prospective study that examined the correlation of CTCs with radiographic findings for disease progression. Serial CTC levels were obtained in patients (n=68) that were starting a new treatment regimen for progressive, radiographically measurable metastatic breast cancer. Blood was collected at baseline and three to four week intervals and radiographic studies were performed in nine to twelve week intervals. Median follow-up was 13.3 months. Patients who had five or more CTCs had 6.3 times the odds of radiographic disease progression when compared with patients who had less than five CTCs. Shorter progression-free survival was observed for patients with five or more CTCs at three to five weeks and at seven to nine weeks after the start of treatment. The CTC result was statistically significantly associated with disease progression for all patients (p<.001). The association was noted to remain strong in patients treated with either chemotherapy or endocrine therapy. Potential limitations of the study include that the study included patients receiving various lines and types of therapy. The subgroup analysis for CTC-imaging correlation was performed by including biologic agents with either chemotherapy or endocrine therapy—it was noted that each group was too small to be analyzed alone.

Nole et al. (2007) conducted a prospective study to evaluate the prognostic significance of CTCs detection in advanced breast cancer patients. The study included 80 patients with inclusion criteria: women with histological diagnosis of breast cancer, evidence of metastatic disease from imaging studies, starting a new line of therapy and/or treated for the advanced disease with a maximum two lines of therapy. The CellSearch system was used to test for circulating tumor cell levels before starting a new treatment and after four, eight weeks and the first clinical evaluation and every two months thereafter. At baseline, 49 patients were found to have ≥ 5 CTCs. The baseline number of CTCs were associated with progression-free survival (hazard ratio [HR] 2.5; 95% confidence interval [CI] 1.2–5.4). The risk of progression for patients with CTCs ≥ 5 at the last available blood draw was five times the risk of patients with 0–4 CTCs at the same time point (HR 5.3; 95% CI 2.8–10.4). At the last available blood draw, patients with rising or persistent CTCs ≥ 5 demonstrated a statistically significant higher risk of progression with respect to patients with CTCs < 5 at both blood draws (HR 6.4; 95% CI 2.8–14.6). The authors noted that these results indicate that elevated CTCs levels measured at any time in the clinical course of a patient with metastatic breast cancer predict an imminent progression and that this analysis represents an additional step in the process of validating this method. There are still unanswered questions regarding the treatment of a patient with low or high levels of CTCs in breast cancer.
Prostate Cancer
Folkersma et al. (2012) reported on a prospective study that analyzed the correlation between circulating tumor cell (CTC) levels and clinicopathologic parameters (prostate-specific antigen [PSA] level, Gleason score, and TNM stage) in patients with metastatic hormone-sensitive prostate cancer (PCa) and to establish its prognostic value in overall survival (OS) and progression-free survival (PFS). The study included three arms: 30 patients with localized PCa; 30 patients with metastatic PCa; and, 30 healthy volunteers. The median follow-up was 42.9 months. A significant positive correlation was demonstrated between the CTC level and all tumor burden markers (PSA and T, N, and M stage; P<.001), except for Gleason score (tau=0.16). A cutoff of ≥4 CTCs/7.5 mL was chosen to distinguish patients with a poor prognosis. This patients had a significantly shorter median OS and PFS (24 compared to 45 months and 7 compared to 44 months, respectively; P<.001). As the CTC level increased, the OS and PFS were noted to decrease. The risk of mortality and progression for the patients with ≥4 CTCs was 4.1 (P=.029) and 8.5 (P<.001) times greater. Multivariate analyses indicated that a CTC of ≥4 was an independent prognostic factor for PFS (hazard ratio 5.9, P<.005). The study is preliminary and needs to be confirmed in larger studies. Further studies need to be conducted to demonstrate the clinical utility of detection of CTC in prostate cancer.

Several observational studies have been published that correlate CTC with disease status and progression in prostate cancer (Goodman, et al. 2009; Okegawa, et al., 2009; Okegawa, et al., 2008; Scher, et al., 2009; Olmos, et al., 2009; Danila, et al., 2007; and Shaffer, et al., 2007; Moreno, et al., 2005). Prospective, large-scale studies will need to be conducted to determine the role of these findings in clinical outcomes.

Colorectal Cancer
Groot Koerkamp et al. (2013) reported on systematic review of studies that investigated the prognostic value of tumor cells in blood (CTCs) or bone marrow (BM) (disseminated tumor cells [DTC]) of patients with resectable colorectal liver metastases or widespread metastatic colorectal cancer (CRC). A total of 16 studies with 1,491 patients were included in the review and the results of 12 studies (1,329 patients) included in the meta-analysis. Eight studies used RT-PCR methodology to detect tumor cells, nine studies applied immunocytochemistry (five with CellSearch) and one study applied both methods. The overall survival (hazard ratio [HR], 2.47; 95 % CI 1.74–3.51) and progression-free survival (PFS) (HR, 2.07; 95 % CI 1.44–2.98) were worse in patients with CTCs. The subgroup of studies with more than 35% CTC-positive patients was the only subgroup with a statistically significant worse PFS. The eight studies that had multivariable analysis identified the detection of CTCs as an independent prognostic factor for survival. Limitations of the study included a considerable degree of interstudy heterogeneity. The study does not demonstrate the clinical utility of CTC detection, or that the detection of CTCs is a predictive factor, or identify patients that may benefit from a specific treatment. Further studies are needed to investigate the clinical utility of detection of CTCs in metastatic colorectal cancer.

Sastre et al. (2012) reported on an ancillary study of 180 patients that was a subset of a phase III study (The Maintenance in Colorectal Cancer trial) that assessed maintenance therapy with single-agent bevacizumab versus bevacizumab plus chemotherapy in patients with metastatic colorectal cancer. The ancillary study was conducted to evaluate CTC count as a prognostic and/or predictive marker for efficacy endpoints. Blood samples were obtained at baseline and after three cycles. CTC enumeration was performed with CellSearch System. The study found that the median progression-free survival (PFS) interval for patients with a CTC count ≥3 at baseline was 7.8 months, as compared to 12.0 months found in patients with a CTC count <3 (p=.0002). The median overall survival (OS) time was 17.7 months for patients with a CTC count >3, compared with 25.1 months for patients with a lower count (p=.0059). After three cycles, the median PFS interval for patients with a low CTC count was 10.8 months, which was noted to be longer than the 7.5 months for patients with a high CTC count (p=.005). The median OS time for patients with a CTC count <3 was significantly longer than for patients with a CTC count ≥3, 25.1 months compared to 16.2 months, respectively (p=.0095). Further studies are needed to identify the role of CTC in treatment of metastatic colorectal cancer.

Thorsteinsson et al. (2011) conducted a review of studies of CTCs in colorectal cancer (CRC). Nine studies were included in the review. Detection rates of CTC in peripheral blood of patients with non-metastatic CRC varied from 4% to 57%. Inclusion criteria included: patients diagnosed with non-metastatic colorectal cancer; CTC detected in peripheral blood samples; pre- and/or post-operative blood samples; and, samples size of more than 99 patients. Seven studies applied RT-PCR and two studies used immunocytochemical methods. Seven studies
found the presence of CTC to be a prognostic marker of poor disease-free survival. The authors concluded that
the presence of CTC in peripheral blood is a potential marker of poor disease-free survival in patients with non-
metastatic CRC and that the low abundance of CTC in non-metastatic CRC needs very sensitive and specific
detection methods. They also noted that an international consensus on choice of detection method and markers
is warranted before incorporating CTC into risk stratification in the clinical setting.

Rahbari et al. (2010) reported on a meta-analysis of studies to assess whether the detection of tumor cells in
blood and bone marrow of patients diagnosed with colorectal cancer (CRC) can be used as a prognostic factor.
Thirty-six studies were included in the review that examined the detection of free blood or bone marrow tumor
cells with patients prognosis and included various methods of techniques (e.g., reverse transcriptase-PCR [RT-
PCR]) and immunologic). The review indicated that the presence of CTCs detected in peripheral blood is of
strong prognostic significance in patients with CRC. There was considerable interstudy heterogeneity noted in
regards to differences in the detection methods, types and numbers of target genes or antigens, sampling site
and time, and in demographic or clinico-pathologic status of patients.

Professional Societies/Organization
For a summary of professional society recommendations/guidelines regarding circulating tumor cells please click
here.

Prostate Cancer Screening and Prognostic Tests

Prostate specific antigen (PSA), an organ-specific marker, is often used as a tumor marker. The higher the level
of PSA at baseline, the higher is the risk for metastatic disease or subsequent disease progression. However, it
is an imprecise marker of risk. Various approaches aimed at improving the performance of PSA in early cancer
detection have been tested, including the measurement of prostate biomarkers. None are clearly more accurate
than total serum PSA levels (National Cancer Institute [NCI], 2016). According to the National Comprehensive
Cancer Network Guideline (NCCN Guidelines™) for Prostate Cancer Early Detection, tests that have been
shown to increase specificity in the post-biopsy state are percent free PSA (%fPSA), 4Kscore (OPKO Health,
Inc., Miami, FL), Prostate Health Index (PHI), (Beckman Coulter, Atlanta, GA), prostate cancer gene 3 (PCA3,
Progensa® PCA3, Gen-Probe, Inc., San Diego, CA) and ConfirmMDx for Prostate Cancer (MDX Health, Irvine,
CA). The NCCN also notes that biomarkers that improve the specificity of detection are not recommended as
firstline screening tests, rather for use in those individuals who wish to further define the probability of high-grade
cancer. Improved specificity post biopsy has been demonstrated in the published-peer-reviewed scientific
literature.

Use of selected biomarkers (i.e., percent free PSA, 4Kscore, PCA3, PHI, ConfirmMDx) is supported by published
professional society guidelines (NCCN, 2016) for the detection of prostate cancer to improve specificity. The
4Kscore, percent free PSA and Prostate Health Index (PHI) tests are considered appropriate when results of the
tests will impact management and there is a PSA >3 ng/mL with or without a previous benign biopsy. PCA3 and
ConfirmMDx are considered to be appropriate when results of testing will impact management, the PSA >3
ng/mL and previous biopsy results are benign or indicate focal high-grade prostatic intraepithelial neoplasia
(PIN). The role of these tests for any other indication or clinical scenario has not been established.

Percent Free PSA (% free PSA): Serum PSA exists in both free form and complexed to a number of protease
inhibitors. Assays for total PSA measure both free and complexed forms. Percent-free PSA may be related to
biologic activity of the tumor. The NCCN (2016) notes that unbound or free PSA, expressed as a ratio of total
PSA is clinically useful with the potential to improve early detection, staging and monitoring of prostate cancer.
According to the NCCN, this test has received widespread clinical acceptance, specifically for patients with
normal digital rectal exams who have previously undergone prostate biopsy because they had a total PSA
(tPSA) level within the diagnostic gray zone.
**4Kscore:** This test combines four prostate-specific kallikrein assay results with clinical information in an algorithm that calculates the individual patient’s percent risk for aggressive prostate cancer. It also considers age, digital rectal exam results and prior biopsy status. According to the manufacturer’s website, the 4Kscore is not indicated for men who have a diagnosis of prostate cancer, are taking or have taken 5-alpha reductase inhibitors within the last 6 months or have recently undergone a prostate procedure within the last 6 months. This test is a laboratory developed test and is not FDA approved. According to the NCCN Guidelines™, the test can be considered for patients prior to biopsy and for those with prior negative biopsy for those thought to be at higher risk for clinically significant prostate cancer. No cut-off threshold has been established for the 4Kscore.

**Progensa® PCA3:** Progensa PCA3 is an in vitro nucleic acid amplification test. The assay measures the concentration of prostate cancer gene 3 (PCA3) and prostate-specific antigen (PSA) RNA (RNA) molecules and calculates the ratio of PCA3 RNA molecules to PSA RNA molecules (PCA3 Score) in post digital rectal exam (DRE) first catch male urine specimens. U.S. Food and Drug Administration (FDA): According to the U.S. Food and Drug Administration (FDA) (2012) it is intended for use in conjunction with other patient information to aid in the decision for repeat biopsy in men 50 years of age or older who have had one or more previous negative prostate biopsies and for whom a repeat biopsy would be recommended by a urologist based on current standard of care, before consideration of Progensa PCA3 Assay results.

**Prostate Health Index (PHI):** This test is a combination of existing tests (Access Hybritech PSA, Access Hybritech free PSA, and Access Hybritech p2PSA, Beckman Coulter, Atlanta, GA) for total PSA, free PSA and proPSA. According to the manufacturer’s website, a proprietary algorithm provides a probability of prostate cancer. PHI results are intended to be used as an aid in distinguishing prostate cancer from benign prostatic conditions in men 50 years of age and older with total PSA results in the 4 – 10 ng/mL range and negative digital rectal examination (DRE) findings. The three assays that make up this test have received FDA approval with numerous supplements.

**ConfirmMDx for Prostate Cancer:** This test is a tissue-based epigenetic assay which aids in the stratification of men being considered for repeat prostate biopsy. The test uses DNA methylation to assess the presence of cancer biomarkers (i.e., GSTP1, APC, RASSF1) in core biopsy tissue samples. ConfirmMDx is a laboratory developed test and is not FDA approved.

**Professional Society/Organizations**
Each of these tests is specifically mentioned in the NCCN Guideline for Prostate Cancer Early Detection as a category 2A recommendation. For additional information regarding professional society recommendations please click here.

**Tumor Tissue-Based Molecular Assays for Prostate Cancer**

The NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines™) for Prostate Cancer (2018) notes that although risk groups, life expectancy estimates and nomograms help inform treatment decisions, there remains uncertainty regarding the risk of disease progression. Several tumor tissue-based molecular assays have been included in the guideline for prostate cancer (2018). The guideline notes that men with low or favorable intermediate risk may consider the use of certain molecular tests (i.e., Decipher®, OncotypeDx Genomic Prostate Score®, Prolaris® Prostate Cancer Test, ProMark Proteomic Prostate Test), which are briefly reviewed in this section of the Coverage Policy.

Although these tests have not been validated by prospective, randomized clinical trial data, retrospective case cohort studies demonstrate that these tests provide prognostic information independent of NCCN risk groups for
men with low or favorable intermediate risk disease, including likelihood of death with conservative management, likelihood of biochemical recurrence after radical prostatectomy or radiotherapy and likelihood of developing metastasis after operation or salvage radiotherapy (NCCN, 2018).

**Decipher® Prostate Cancer Classifier Assay (GenomeDx, San Diego, CA):** This test is a 22 biomarker genomic expression classifier assay which uses formalin-fixed paraffin embedded (FFPE) tissue from a radical prostatectomy specimen to predict the probability of metastasis and tumor aggressiveness. Decipher is listed as a Category 2B recommendation in the NCCN Practice Guidelines in Oncology for Prostate Cancer as an option following radical prostatectomy with PSA persistence/recurrence defined as failure of PSA to fall to undetectable levels (PSA persistence) or undetectable PSA after radical prostatectomy with a subsequent PSA that increases on two or more determinations (PSA recurrence). The Guideline also notes that Decipher may be used in men with low-risk prostate cancer, defined as T1-T2a disease, Gleason score ≤6/grade group 1 and a PSA <10ng/mL or those with favorable intermediate-risk disease, defined as T2b-Tc disease, Gleason score 3+4=7/grade group 2, PSA 10-20 ng/mL and percentage of positive biopsy cores <50%.

**OncotypeDx Genomic Prostate Score (Genomic Health®, Redwood City, CA):** This test is a genomic classifier test measuring the activity of 17 genes to predict clinical risk and tumor aggressiveness. OncotypeDx Prostate uses FFPE tissue from a prostate biopsy specimen. The NCCN Practice Guidelines in Oncology for Prostate Cancer notes that men with low or favorable intermediate risk prostate cancer may consider the use of this test after prostate biopsy for low or favorable intermediate risk prostate cancer when there is a ≥ 10 years life expectancy and the individual is a candidate for active surveillance or definitive therapy.

**Prolaris® Prostate Cancer Test (Myriad Genetic Laboratories, Inc., Salt Lake City, UT):** This test is a gene expression classifier risk stratification tool designed to measure the expression level of 31 genes in a prostate cancer tumor biopsy tissue, in conjunction with clinical parameters such as the Gleason score and PSA. The NCCN Practice Guidelines in Oncology for Prostate Cancer notes that men with low or favorable intermediate risk prostate cancer may consider the use of this test post prostate biopsy for low or favorable intermediate risk prostate cancer when there is a ≥ 10 years life expectancy and the individual is a candidate for active surveillance or definitive therapy.

**ProMark Proteomic Prognostic Test (Metamark, Waltham, MA):** This test is a prognostic assay that measures the signal intensity of eight protein biomarkers in FFPE prostate biopsy tissue. Using a proprietary algorithm the test generates a risk score indicating the likelihood of having high-risk disease. The NCCN NCCN Practice Guidelines in Oncology for Prostate Cancer notes that men with low or favorable intermediate risk prostate cancer may consider the use of this test post prostate biopsy for low or favorable intermediate risk prostate cancer when there is a ≥ 10 years life expectancy and the individual is a candidate for active surveillance or definitive therapy.

**Hematologic Cancer and Myeloproliferative and Myelodysplastic Disease**

**Polycythemia Vera (PV), Essential Thrombocythemia (ET) and Primary Myelofibrosis (PMF)**

Identification of the JAK2 V617F mutation in individuals with polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) may aid in diagnosis based on diagnostic criteria for each of these diseases. For some individuals with PV, JAK2 exon 12 mutation testing may also be of benefit in disease management. Likewise genetic testing for MPL common variants and targeted mutation analysis of CALR exon 9 may be appropriate to aid in the diagnosis and management of ET and PMF. According to 2016 World Health Organization (WHO) criteria (Arber, 2016), ASXL1, EZH2, TET2, IDH1/IDH2, SRSF2 and SF3B1 mutation analysis may aid in diagnosis of PMF.
Chronic Myelogenous Leukemia and Philadelphia Chromosome Positive (Ph+) Acute Lymphoblastic Leukemia Mutation Testing

Specific mutations in the Breakpoint Cluster Region-Abelson (BCR-ABL) gene have been shown to confer resistance to imatinib both in vitro and in vivo, by affecting the binding of the drug to the tyrosine kinase enzyme (AHRQ, 2010). Of interest is the T315-I mutation which is thought to be resistant to all current TKI therapy. The mutation frequency in imatinib resistant patients with CML ranges between 2% and 20%, with variability related to detection methods as well as patient cohort characteristics and treatment. T315I mutation frequency appears to be greater in patients with Philadelphia chromosome-positive (Ph+) ALL and likely increases with the continuation of TKI treatment (Nicolini, 2009). The detection of mutations of the BCR-ABL gene has been proposed with potential impact on diagnosis and management decisions (Agency for Healthcare Research and Quality [AHRQ], 2010; National Cancer Institute [NCI], 2015; Najfeld, 2012; National Institute for Clinical Excellence [NICE], 2002). Evidence in the published, peer-reviewed scientific literature also supports the usefulness of testing for BCR-ABL resistance or inhibition.

Real-time quantitative PCR (RQ-PCR) is by far the most sensitive method. It provides an accurate measure of the total leukemia cell mass and the degree to which breakpoint cluster region-Abelson (BCR-ABL) transcripts are reduced by therapy, and correlates with progression-free survival. Current international recommendations for optimal molecular monitoring of patients receiving imatinib treatment include an RQ-PCR assay expressing the BCR-ABL transcript levels, which is predictive of prognosis (Bhatia, 2012; Najfeld, 2012). Molecular responses at 12 and 18 months are also predictive of long-term outcome (Bhatia, 2012). In acute lymphocytic leukemia (ALL), because many patients have a different fusion protein from the one found in chronic myelogenous leukemia (CML), the BCR-ABL gene may be detectable only by pulsed-field gel electrophoresis or reverse-transcriptase polymerase chain reaction (RT-PCR). These tests should be performed whenever possible in patients with ALL, especially those with B-cell lineage disease (NCI, 2015a).

Although certain BCR-ABL mutations may be associated with TKI therapy resistance, sensitivity and specificity values in outcome studies are not suggestive of strong predictive ability, with the exception of the T315-I mutation. Early identification of this mutation may allow for alternative treatment regimens including increased dose scheduling and drug selection. Data in the published peer-reviewed scientific literature supports the clinical utility of testing for the presence of the T315-I mutation. The clinical utility of testing for other mutations to determine TKI resistance has not been established.

Literature Review

Several studies have reported associations between variations of BCR-ABL and response to drug therapy. AHRQ (2010) performed a systematic review of the published literature regarding variations of the BCR-ABL1 fusion gene and response to imatinib, dasatinib, and nilotinib in CML. Thirty-one studies were analyzed for outcomes of interest including overall survival and cancer specific survival; progression-free or event-free survival (as defined by each study); and treatment failure. Typically, treatment failure is defined as absence of hematologic, cytogenetic, or molecular response to treatment, according to various criteria. Data was analyzed for first-, second-, and third- line TKI therapy. Second-line TKI therapy studies (four publications) demonstrated sensitivity and specificity ranges of 0.35 to 0.83 and from 0.58 to 1.00, respectively, for high-dose imatinib and imatinib-based combination. These studies were small, the calculated sensitivity and specificity values have wide confidence intervals, and a range of different mutations was identified in each of them. No robust conclusions could be made. Eight studies (nine publications) pertained to dasatinib; some had overlapping populations. Sensitivities and specificities ranged from 0.27 to 0.90 and from 0.14 to 0.87, respectively. A lack of predictive ability is suggested. For nilotinib, three studies had relevant data. Sensitivity ranged from 0.56 to 0.71 and specificity ranged from 0.42 to 0.56 for all identified mutations. Only one included study reviewed overall survival (OS). No statistically significant differences in the time-to-death among patients with, versus without mutations were found. When any breakpoint cluster region- Abelson (BCR-ABL1) mutation was considered, almost all studies reported sensitivity and specificity values that are not suggestive of strong predictive ability. The Agency for Healthcare Research and Quality (AHRQ) notes that no study explicitly reported details on changes in treatment plans before or after testing.

AHRQ determined that the presence of any BCR-ABL mutation does not appear to differentiate response to tyrosine kinase inhibitor (TKI) treatment (i.e., imatinib, dasatinib, nilotinib). AHRQ also notes that the majority of
Evidence pertains to the short term surrogate outcomes of hematologic, cytogenetic or molecular response. Data on overall or progression-free survival are sparse. There is consistent evidence that presence of the relatively rare T315-I mutation can predict TKI treatment failure, mainly in terms of hematologic and cytogenetic response.

Jabbour et al. (2009) studied 169 patients with chronic myelogenous leukemia (CML) after imatinib failure. The goals of the study were to investigate whether in vitro sensitivity of kinase domain mutations could be used to predict the response to therapy as well as the long-term outcome of patients receiving second-generation TKIs after imatinib failure. Treatment failure was defined as loss of a cytogenetic, or complete hematologic response (CHP), or failure to achieve a CHR or any hematologic response (for patients in accelerated phase or blast phase after 3 months of therapy, or persistence of 100% Philadelphia chromosome (Ph)-positive metaphases after 6 months of therapy, or more than or equal to 35% after 12 months). Fifty-seven patients (66%) had received prior therapy with interferon-alpha before the start of imatinib; 29 (34%) had received imatinib as their first-line therapy for CML. Mutations were detected by cDNA sequencing for mutations in the kinase domain of BCR-ABL before a change to dasatinib or nilotinib in 86 patients. Ninety-four mutations were identified in 86 patients with imatinib failure. Seven patients harbored more than 1 mutation. There was no difference in patient characteristics between those with mutations at the time of imatinib failure versus those with no mutations. Forty-one patients received dasatinib and 45 received nilotinib after developing failure to imatinib therapy. Hematologic and cytogenetic response rates were similar for patients without or with KD mutations. After a median follow-up of 23 months, 48 (58%) of patients without baseline mutations were alive compared with 52 (60%) with any mutation.

Nicolini et al. (2009) reported the results of a retrospective observational study of 222 patients with CML in chronic-phase, accelerated-phase, or blastic-phase and Philadelphia chromosome-positive (Ph+) ALL patients with the BCR-ABL T315I mutation. After T315I mutation detection, second-generation TKIs were used in 56% of cases, hydroxyurea in 39%, imatinib in 35%, cytarabine in 26%, MK-0457 in 11%, stem cell transplantation in 17%, and interferon-alpha in 6% of cases. Median overall survival from T315I mutation detection was 22.4, 28.4, 4.0, and 4.9 months, and median progression-free survival was 11.5, 22.2, 1.8, and 2.5 months, respectively, for chronic phase, accelerated phase, blastic phase, and Ph(+) ALL patients. These results suggest that survival of patients harboring a T315I mutation is dependent on disease phase at the time of mutation detection.

In an earlier study by Jabbour et al. (2006) 171 patients were screened for mutations after failing TKI therapy with a median follow-up of 38 months from start of therapy. Sixty-six mutations impacting 23 amino acids in the BCR-ABL oncogene were identified in 62 (36%) patients. Factors associated with the development of mutations were older age, previous interferon therapy and accelerated or blast phase at the start of TKI therapy. By multivariate analysis, factors associated with a worse survival were development of clonal evolution and a higher percentage of peripheral blood basophils. The presence of a BCR-ABL kinase domain mutation had no impact on survival. When survival was measured from the time therapy started, non-P-loop mutations were associated with a shorter survival than P-loop mutations. The authors concluded that BCR-ABL P-loop mutations were not associated with a worse outcome. This study suggests that outcomes of individuals who fail TKI therapy may be influenced by multiple factors.

Nicolini and colleagues (2006) retrospectively analyzed the predictive impact of 94 breakpoint cluster region (BCR)-Abelson (ABL) kinase domain mutations found in 89 protein tyrosine kinase inhibitor (TKI) resistant chronic myelogenous leukemia (CML) individuals. With a median follow-up of 39 months, overall survival was worse for P-loop and another point mutation (T315-I), but not for other BCR-ABL mutations. For individuals in chronic phase only, analysis demonstrated a worse overall survival for P-loop and worse progression free survival for T315-I mutations.

**Professional Societies/Organizations**

For a summary of professional society recommendations/guidelines regarding BCR-ABL mutation analysis please click [here](#).

**Occult Neoplasms**
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).

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. 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).

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 (voltage-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).

**Solid Tumor Cancers**

Molecular testing for the following tumor markers has been proposed to direct treatment and disease management. However, there is insufficient evidence in the published, peer-reviewed scientific literature to demonstrate the clinical utility of tumor analysis and/or gene expression profiling for the following tumor types. Further, consensus support in the form of published professional society guidelines is lacking.

| Tumor Type                              |  |
|-----------------------------------------|  |
| Anal carcinoma                          | Malignant mesothelioma |
| Basal cell carcinoma                    | Non-Hodgkin lymphoma |
| Bone cancer                             | Penile cancer |
| Cancer of unknown origin/unknown primary| Testicular cancer |
| Cervical cancer                         | Tracheal cancer |
| Head and neck cancer                    | Esophageal cancer |
| Hepatobiliary cancer                    | Squamous cell carcinoma of the skin |
| Hodgkin lymphoma                        | Renal/kidney cancer |

**Professional Societies/Organizations**

For a summary of professional society recommendations/guidelines regarding molecular testing for solid tumor cancers please click [here](#).

**Other Tumor Profile Testing**

**Topographic Genotyping**
Topographic genotyping refers to a method of mutational analysis that incorporates minute tumor samples selected according to histopathologic considerations, polymerase chain reaction (PCR) amplification and direct sequencing. The mutational alterations that are found are then correlated with the histology of the tumor. It has been proposed that the results of this testing will provide predictive information that will influence the management of certain cancers.

Studies comparing topographic genotyping with established testing methods are lacking. There do not appear to be prospective studies published in the peer-reviewed medical literature that focus on the clinical validity, the clinical utility of the test or the impact of the test on clinical outcomes

**Literature Review**

High-quality prospective controlled studies informing the clinical validity and clinical utility of topographic genotyping tests are lacking in the published, peer-reviewed scientific literature. Studies generally focus on the association of the topographic genotyping results with tumor characteristics (Al-Haddad, et al., 2014; Al-Haddad et al., 2013; Malhotra et al., 2014; Panarelli et al., 2012; Khalid, et al., 2009).

A technology assessment and systematic review regarding topographic genotyping with PathFinderTG was commissioned by Centers for Medicare and Medicaid Services (CMS) and conducted by the Tufts Evidence-based Practice Center for the Agency for Healthcare Research and Quality (AHRQ) (Trikalinos TA, et al., 2010). The review included studies evaluating the patented technology, specifically those using loss of heterozygosity (LOH) analysis. LOH is a frequent genetic alteration that is found in many cancers. It is thought that LOH alterations may have prognostic significance. Fifteen studies were included—these pertained to: lung cancer (n=4); pancreatic and biliary tree tumors (n=4); hepatocellular carcinoma (n=4); gliomas, thyroid tumors, lacrimal gland tumors and mucinous tumors of the appendix (n=1 for each). The sample size in the studies ranged from 11 to 103. The review identified no studies regarding the analytic validity of LOH based topographic genotyping with PathFinderTG. The studies were retrospective in design and utilized available archival tissue blocks. One study, molecular profiles of gliomas and reactive gliosis were determined retrospectively and they were used prospectively on 16 diagnostically challenging cases of reactive gliosis versus glial tumors. There were no studies found that evaluated whether the use of LOH based topographic genotyping with PathFinderTG affects patient outcomes. There were no studies identified that compared LOH based topographic genotyping with PathFinderTG with conventional pathology. The review found that all studies are small, they have important methodological limitations, and they do not address patient-relevant outcomes.

**Professional Societies/Organization**

For a summary of professional society recommendations/guidelines regarding topographic genotyping please click [here](#).

**The American Board of Internal Medicine’s (ABIM) Foundation Choosing Wisely® Initiative (2014):** No relevant statements.

**Use Outside of the US**

For a summary of recommendations/guidelines from professional societies outside of the US please click [here](#).

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**Appendix A**

**PROFESSIONAL SOCIETY/ORGANIZATION RECOMMENDATIONS/GUIDELINES**

**GENE EXPRESSION CLASSIFIER TESTS**

American Society of Clinical Oncology ([ASCO], 2016): In addition to estrogen and progesterone receptors and human epidermal growth factor receptor 2, the panel found sufficient evidence of clinical utility for the biomarker assays Oncotype DX, EndoPredict, PAM50, Breast Cancer Index, and urokinase plasminogen activator and plasminogen activator inhibitor type 1 in specific subgroups of breast cancer. No biomarker except for estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 was found to guide choices of specific treatment regimens. Treatment decisions should also consider disease stage, comorbidities, and patient preferences.

A NICE guidance document titled “Gene Expression Profiling and Expanded Immunohistochemistry Tests for Guiding Adjuvant Chemotherapy Decisions in Early Breast Cancer Management: MammaPrint, Oncotype DX, IHC4 and Mammostrat (2013) notes that Oncotype DX is recommended as an option for guiding adjuvant chemotherapy decisions for people with oestrogen receptor positive (ER+), lymph node negative (LN−) and human epidermal growth factor receptor 2 negative (HER2−) early breast cancer if the person is at intermediate risk and information provided by Oncotype DX is likely to help in predicting the course of the disease and would assist in making chemotherapy decisions. The guideline recommends MammaPrint, IHC4 and Mammostrat for use in research in people with ER+, LN− and HER2− early breast cancer, to collect evidence about potentially important clinical outcomes and to determine the ability of the tests to predict the benefit of chemotherapy. NICE notes that the tests are not recommended for general use in these people because of uncertainty about their overall clinical benefit and consequently their cost effectiveness.

MammaPrint® 70-Gene Breast Cancer Recurrence Assay
American Society of Clinical Oncology (ASCO, 2017): On behalf of ASCO, Krop et al. published a focused update: Use of Biomarkers to Guide Decisions on Adjuvant Systemic Therapy for Women With Early-Stage Invasive Breast Cancer which addressed the use of MammaPrint to guide decisions on the use of adjuvant systemic therapy. ASCO recommends the following:

- If a patient has ER/PgR–positive, HER2-negative, node-negative, breast cancer, the MammaPrint assay may be used in those with high clinical risk per MINDACT categorization to inform decisions on withholding adjuvant systemic chemotherapy due to its ability to identify a good prognosis population with potentially limited chemotherapy benefit (Type: evidence based; Evidence quality: high; Strength of recommendation: strong).
- If a patient has ER/PgR–positive, HER2-negative, node-negative, breast cancer, the MammaPrint assay should not be used in those with low clinical risk per MINDACT categorization to inform decisions on withholding adjuvant systemic chemotherapy, because women in the low clinical risk category had excellent outcomes and did not appear to benefit from chemotherapy even with a genomic high-risk cancer (Type: evidence based; Evidence quality: high; Strength of recommendation: strong).
- If a patient has ER/PgR–positive, HER2-negative, node-positive, breast cancer, the MammaPrint assay may be used in patients with one to three positive nodes and at high clinical risk per MINDACT categorization to inform decisions on withholding adjuvant systemic chemotherapy due to its ability to identify a good prognosis population with potentially limited chemotherapy benefit. However, such patients should be informed that a benefit of chemotherapy cannot be excluded, particularly in patients with greater than one involved lymph node (Type: evidence based; Evidence quality: high; Strength of recommendation: moderate).
- Recommendation 1.2.2: (update of 2016 recommendation 1.7): If a patient has ER/PgR–positive, HER2-negative, node-positive, breast cancer, the MammaPrint assay should not be used in patients with one to three positive nodes and at low clinical risk per MINDACT categorization to inform decisions on withholding adjuvant systemic chemotherapy. There are insufficient data on the clinical utility of MammaPrint in this specific patient population (Type: informal consensus; Evidence quality: low; Strength of recommendation: moderate).
- Recommendation 1.3: (update of 2016 recommendation 1.8): If a patient has HER2-positive breast cancer, the clinician should not use the MammaPrint assay to guide decisions on adjuvant systemic therapy. Additional studies are required to address the role of MammaPrint in patients with this tumor subtype who are also receiving HER2-targeted therapy (Type: informal consensus; Evidence quality: low; Strength of recommendation: moderate).
- Recommendation 1.4: (update of 2016 recommendation 1.9): If a patient has ER/PgR negative and HER2-negative (triple negative) breast cancer, the clinician should not use the MammaPrint assay to guide decisions on adjuvant systemic chemotherapy (Type: informal consensus; Evidence quality: insufficient; Strength of recommendation: strong).
Medical Coverage Policy: 0520

National Comprehensive Cancer Network™ (NCCN™): The Clinical Practice Guidelines in Oncology for Breast Cancer (V2.2 2017) notes that there are prognostic multigene assays that may be considered to help assess risk of recurrence but have not been validated to predict response to chemotherapy.

Oncotype DX Assay
Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group (2016): A published recommendation for the EGAPP notes there is insufficient evidence to recommend for or against the use of Oncotype DX testing to guide chemotherapy treatment decisions in women with hormone receptor–positive, lymph node–negative, or lymph node–positive early breast cancer who are receiving endocrine therapy. Regarding clinical utility, the EGAPP notes there is evidence from prospective retrospective studies that the test predicts benefit from chemotherapy, and there was adequate evidence that the use of Oncotype DX gene expression profiling in clinical practice changes treatment decisions regarding chemotherapy. However, no direct evidence was found that the use of Oncotype DX testing leads to improved clinical outcomes.

National Comprehensive Cancer Network™ (NCCN™): The Clinical Practice Guidelines in Oncology for Breast Cancer (V2.2 2016) has identified use of the 21 gene RT-PCR as a category 2A level of evidence and consensus recommendation for a woman with pT1, pT2, or pT3 or pN0 or pN1mi (≤2mm axillary node metastasis) or node positive disease (one or more metastasis >2 mm to one or more ipsilateral axillary lymph nodes). The Guideline also notes many other multi-gene or multi-gene expression assay systems have been developed, however, the 21-gene assay has been best validated for its use as a prognostic test as well as in predicting who is most likely to systemic chemotherapy.

Spanish Society of Pathology (SEAP) and the Spanish Society of Medical Oncology (SEOM): In a joint guideline for biomarker testing in colon cancer published by Garcia-Alfonso on behalf of SEAP/SEOM, the authors noted although Oncotype DX gene expression signature has been shown to have prognostic value, no consensus yet exists 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 (2012).

Prosigna® Breast Cancer Prognostic Gene Signature Assay
American Society of Clinical Oncology ([ASCO], 2016): On behalf of ASCO, Harris et al. published recommendations titled Use of Biomarkers to Guide Decisions on Adjuvant Systemic Therapy for Women With Early-Stage Invasive Breast Cancer: American Society of Clinical Oncology Clinical Practice Guideline Summary. Regarding the PAM50 risk of recurrence score ASCO notes that if a patient has ER/PgR-positive, HER2 negative (node negative) breast cancer, a clinician may use this assay in conjunction with other clinicopathologic variables to guide decisions on adjuvant systemic therapy. (High Quality Evidence; Strong Recommendation)

National Comprehensive Cancer Network™ (NCCN™): The NCCN Guideline for Breast Cancer (V2.2016) notes that other prognostic multigene assays may be considered to help assess risk of recurrence but have not been validated to predict response to chemotherapy. The Prediction Analysis of Microarray 50 (PAM50) can be used to predict prognosis among postmenopausal women with hormone-positive breast cancer.

CIRCULATING WHOLE TUMOR CELL MARKERS
American Society of Clinical Oncology (ASCO, 2016): A Guideline on the Use of Biomarkers to Guide Decisions on Systemic Therapy for Women With Metastatic Breast Cancer notes for patients already receiving systemic therapy for metastatic breast cancer, decisions on changing to a new drug or regimen or discontinuing treatment should be based on clinical evaluation, judgment of disease progression or response, and the patient’s goals for care. The Guideline also notes there is no evidence at this time that changing therapy based solely on circulating biomarker results improves health outcomes, quality of life, or cost effectiveness.

Prostate Cancer Screening and Prognostic Tests
American Urological Association (2013): In the guideline for “Early Detection of Prostate Cancer”, Carter et al. (2013) note that the literature supporting the efficacy of DRE, PSA derivatives and isoforms (e.g. free PSA, -2proPSA, prostate health index, hK2, PSA velocity or PSA doubling time) and novel urinary markers and biomarkers (e.g. PCA3) for screening with the goal of reducing prostate cancer mortality provide limited evidence to draw conclusions. While some data suggest use of these secondary screening tools may reduce unnecessary
biopsies (i.e. reduce harms) while maintaining the ability to detect aggressive prostate cancer (i.e. maintain the benefits of PSA screening), more research is needed to confirm this. However, the likelihood of a future population-level screening study using these secondary screening approaches is highly unlikely at least in the near future. The authors further note that the Guideline focuses only on the efficacy of PSA screening for the early detection of prostate cancer and not secondary tests often used after screening to determine the need for a prostate biopsy or a repeat prostate biopsy (e.g., PSA isoforms, PCA3, imaging).

**National Comprehensive Cancer Network (NCCN Guidelines™):** The Guideline for Prostate Cancer Early Detection (V2.2016) notes that PSA derivatives and other assays potentially improve the specificity of testing and may diminish the probability of unnecessary biopsies. Several biomarker tests have the goals of refining selection for biopsies, decreasing unnecessary biopsies and increasing the specificity of cancer detection, without missing a substantial number of higher-grade (Gleason ≥ 7) cancers. These tests may be especially useful in men with PSA levels between 3 and 10 ng/mL.

Under indications for biopsy: Percent free PSA, 4KScore or PHI are noted as second line tests for a PSA >3 ng/mL. In a corresponding footnote the NCCN notes that biomarkers that improve specificity of detection are not recommended as first-line screening tests. However, some may wish to further define the probability of high-risk cancer. A percent free PSA <10%, PHI>35 or 4KScore are potentially informative in patient who have never undergone biopsy or after a negative biopsy; a PCA3 score >35 is potentially informative after a negative biopsy.

Regarding the management of biopsy results, NCCN recommends that percent free PSA, 4KScore, PHI, PCA3 or ConfirmMDx be considered for men with focal high-grade prostatic intraepithelial neoplasia (PIN) and those with a benign biopsy result. In a corresponding footnote NCCN notes that it is well known that a negative biopsy does not preclude a diagnosis of prostate cancer on subsequent biopsy. Tests that improve specificity in the post-biopsy state-including 4KScore, PHI, percent free PSA, PCA3 and ConfirmMDx-should be considered in patients thought to be higher risk despite a negative biopsy.

**BCR-ABL MUTATION ANALYSIS**

**National Cancer Institute (NCI):** Regarding BCR-ABL mutation analysis in individuals with chronic myelogenous leukemia (CML), the NCI notes “In case of treatment failure or suboptimal response, patients should undergo BCR/ABL kinase domain mutation analysis to help guide therapy with the newer tyrosine kinase inhibitors or with allogeneic transplantation (2016)

**National Comprehensive Cancer Network™ (NCCN™):** Regarding kinase domain mutation testing, the NCCN Guideline for Chronic Myeloid Leukemia notes kinase domain mutation analysis is recommended in chronic phase CML if there is inadequate initial response at three and six months or less than complete cytogenetic response at 12-18 months, any sign of loss of response, increase in BCR-ABL transcript levels and loss of minimal molecular response (MMR), and disease progression to accelerated or blast phase (V1.2017).

The NCCN Guideline for Ductal Carcinoma in Situ does not support routine CYP2D6 genotype testing for women being considered for tamoxifen therapy (V2.2017).

**TUMOR MARKERS FOR SOLID TUMOR CANCERS**

**American Association of Clinical Endocrinologist (AACE):** In the update of 2010 guidelines for the management of thyroid nodules (2016) the AACE stated that molecular testing should be considered to complement, not replace cytologic evaluation when the results are expected to influence clinical management. As a general rule molecular testing is not recommended in nodules with established benign or malignant cytologic characteristics. Regarding testing of indeterminate nodules the AACE notes because of the insufficient evidence and the limited follow-up, we do not recommend either in favor of or against the use of gene expression classifiers (GECs) for cytologically indeterminate nodules. Regarding use of mutation testing to guide to the extent of surgery the AACE notes with the exception of mutations such as BRAFV600E that have a PPV approaching 100% for papillary thyroid carcinoma (PTC), evidence is insufficient to recommend in favor of or against its use.

**American Association for Endocrine Surgeons:** In a summary statement on the utility of molecular marker testing in thyroid cancer, Yip, et al. 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 (2010).

American Cancer Society ((ACS), 2017): In a discussion of bladder cancer and tumor markers, the ACS stated that NMP22 BladderChek®, bladder tumor-associated antigen (BTA), Immunocyt™ and Urovysion™ are new tests that look for substances in the urine that might indicate bladder cancer. At this time the tests are used mainly to look for bladder cancer in people who already have signs or symptoms of cancer, or in people who have had a bladder cancer removed to check for cancer recurrence. Further research is needed before these or other newer tests are proven useful as screening tests.

American College of Obstetricians and Gynecologists (2016): A Practice Bulletin on Evaluation and Management of Adnexal Masses notes that serum biomarker panels may be used as an alternative to CA 125 level alone in determining the need for a referral or consultation with a gynecologist oncologist when an adnexal mass requires surgery. (Level C recommendation, based primarily on consensus and expert opinion).

American Society of Colon & Rectal Surgeons (ASCRS): In practice parameters for anal squamous neoplasms, ASCRS (2012) noted 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.

American Thyroid Association (ATA): The 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”.

Ferris et al. (2015) published a statement on behalf of the ATA regarding “Surgical Application of Molecular Profiling for Thyroid Nodules: Current Impact on Perioperative Decision Making”. The ATA notes the current gene expression classifier application is to enhance the accuracy of the cytologically indeterminate categories of atypia of uncertain significance/follicular lesion of undetermined significance (AUS/FLUS and follicular neoplasm/suspicious for follicular neoplasm (FN). A benign gene expression classifier (GEC) result may be used to recommend observation and avoid a diagnostic lobectomy, especially in the absence of clinical or sonographic suspicion of malignancy. In the presence of clinical or sonographic suspicion for malignancy, and/or when the local prevalence of malignancy exceeds the 25% reported, diagnostic lobectomy is still warranted. Standard application of the GEC for all indeterminate thyroid nodules would result in only a 7.2% decrease in thyroidectomy volume.

Australia and New Zealand Horizon Scanning Networks (ANZHSN)/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”.

College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology (2013): A joint guideline regarding Molecular Testing Guideline for Selection of Lung Cancer Patients for EGFR and ALK Tyrosine Kinase Inhibitors (Lindeman et al.) recommends
that EGFR molecular testing should be used to select patients for EGFR-targeted and ALK-targeted TKI therapy. EGFR and ALK testing is not recommended in lung cancers that lack any adenocarcinoma component, such as pure squamous cell carcinomas, pure small cell carcinomas, or large cell carcinomas lacking any immunohistochemistry (IHC). To determine EGFR and ALK status for initial treatment selection, primary tumors or metastatic lesions are equally suitable for testing.

Ministry of Health, Singapore: A cancer screening clinical practice guideline 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.

TOPOGRAPHIC GENOTYPING

American Gastroenterological Association Institute: A Guideline on the Diagnosis and Management of Asymptomatic Neoplastic Pancreatic Cysts notes, that molecular techniques to evaluate pancreatic cysts remain an emerging area of research and the diagnostic utility of these tests is uncertain (Vege, et al., 2015).

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.

General Criteria for Somatic Mutation Genetic Testing

Considered Medically Necessary when criteria in the applicable policy statements listed above are met:

<table>
<thead>
<tr>
<th>CPT® Codes</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>81210</td>
<td>BRAF (B-Raf proto-oncogene, serine/threonine kinase) (eg, colon cancer, melanoma), gene analysis, V600 variant(s)</td>
</tr>
<tr>
<td>81235</td>
<td>EGFR (epidermal growth factor receptor) (eg, non-small cell lung cancer) gene analysis, common variants (eg, exon 19 LREA deletion, L858R, T790M, G719A, G719S, L861Q)</td>
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<tr>
<td>81245</td>
<td>FLT3 (fms-related tyrosine kinase 3) (eg, acute myeloid leukemia), gene analysis, internal tandem duplication (ITD) variants (ie, exons 14, 15)</td>
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<td>81272</td>
<td>KIT (v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog) (eg, gastrointestinal stromal tumor [GIST], acute myeloid leukemia, melanoma), gene analysis, targeted sequence analysis (eg, exons 8, 11, 13, 17, 18)</td>
</tr>
<tr>
<td>81273</td>
<td>KIT (v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog) (eg, mastocytosis), gene analysis, D816 variants(s)</td>
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<td>81288</td>
<td>MLH1 (mutL homolog 1, colon cancer, nonpolyposis type 2) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; promoter methylation analysis</td>
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<td>81292</td>
<td>MLH1 (mutL homolog 1, colon cancer, nonpolyposis type 2) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; full sequence analysis</td>
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<td>MLH1 (mutL homolog 1, colon cancer, nonpolyposis type 2) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; known familial variants</td>
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<tr>
<td>81294</td>
<td>MLH1 (mutL homolog 1, colon cancer, nonpolyposis type 2) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; duplication/deletion variants</td>
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<td>81299</td>
<td>MSH6 (mutS homolog 6 [E. coli]) (eg, hereditary nonpolyposis colorectal cancer, Lynch syndrome) gene analysis; known familial variants</td>
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<tr>
<td>81301</td>
<td>Microsatellite instability analysis (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) of markers for mismatch repair deficiency (eg, BAT25, BAT26), includes comparison of neoplastic and normal tissue, if performed</td>
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<tr>
<td>81334</td>
<td>RUNX1 (runt related transcription factor 1) (eg, acute myeloid leukemia, familial platelet disorder with associated myeloid malignancy), gene analysis, targeted sequence analysis (eg,</td>
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<tr>
<td>Code</td>
<td>Description</td>
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<tr>
<td>81401</td>
<td>Molecular pathology procedure, Level 2 (eg, 2-10 SNPs, 1 methylated variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat)</td>
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<tr>
<td>81404</td>
<td>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)</td>
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<tr>
<td>81406</td>
<td>Molecular pathology procedure, Level 7 (eg, analysis of 11-25 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 26-50 exons, cytogenomic array analysis for neoplasia)</td>
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<tr>
<td>81545</td>
<td>Oncology (thyroid), gene expression analysis of 142 genes, utilizing fine needle aspirate, algorithm reported as a categorical result (eg, benign or suspicious)</td>
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<tr>
<td>82105</td>
<td>Alpha-fetoprotein (AFP); serum</td>
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<tr>
<td>82232</td>
<td>Beta-2 microglobulin</td>
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<tr>
<td>82308</td>
<td>Calcitonin</td>
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<tr>
<td>82378</td>
<td>Carcinoembryonic antigen (CEA)</td>
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<td>83497</td>
<td>Hydroxyindolacetic acid, 5-(HIAA)</td>
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<td>83876</td>
<td>Myeloperoxidase (MPO)</td>
</tr>
<tr>
<td>83950</td>
<td>Oncoprotein; HER-2/neu</td>
</tr>
<tr>
<td>84152</td>
<td>Prostate specific antigen (PSA); complexed (direct measurement)</td>
</tr>
<tr>
<td>84153</td>
<td>Prostate specific antigen (PSA); total</td>
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<tr>
<td>84154</td>
<td>Prostate specific antigen (PSA); free</td>
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<tr>
<td>84432</td>
<td>Thyroglobulin</td>
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<tr>
<td>84702</td>
<td>Gonadotropin, chorionic (hCG); quantitative</td>
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<tr>
<td>84703</td>
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<tr>
<td>84704</td>
<td>Gonadotropin, chorionic (hCG); free beta chain</td>
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<tr>
<td>86294</td>
<td>Immunoassay for tumor antigen, qualitative or semiquantitative (eg, bladder tumor antigen)</td>
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<td>86300</td>
<td>Immunoassay for tumor antigen, quantitative; CA 15-3 (27.29)</td>
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<td>86301</td>
<td>Immunoassay for tumor antigen, quantitative; CA 19-9</td>
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<tr>
<td>86304</td>
<td>Immunoassay for tumor antigen, quantitative; CA 125</td>
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<tr>
<td>86316</td>
<td>Immunoassay for tumor antigen, other antigen, quantitative (eg, CA 50, 72-4, 549), each</td>
</tr>
<tr>
<td>86386</td>
<td>Nuclear Matrix Protein 22 (NMP22), qualitative</td>
</tr>
<tr>
<td>88120</td>
<td>Cytopathology, in situ hybridization (eg, FISH), urinary tract specimen with morphometric analysis, 3-5 molecular probes, each specimen; manual</td>
</tr>
<tr>
<td>88121</td>
<td>Cytopathology, in situ hybridization (eg, FISH), urinary tract specimen with morphometric analysis, 3-5 molecular probes, each specimen; using computer-assisted technology</td>
</tr>
<tr>
<td>88271</td>
<td>Molecular cytogenetics; DNA probe, each (eg, FISH)</td>
</tr>
<tr>
<td>88274</td>
<td>Molecular cytogenetics; interphase in situ hybridization, analyze 25-99 cells</td>
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<tr>
<td>88275</td>
<td>Molecular cytogenetics; interphase in situ hybridization, analyze 100-300 cells</td>
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<tr>
<td>88342</td>
<td>Immunohistochemistry or immunocytochemistry, per specimen; initial single antibody stain procedure</td>
</tr>
<tr>
<td>88360</td>
<td>Morphometric analysis, tumor immunohistochemistry (eg, Her-2/neu, estrogen receptor/progesterone receptor), quantitative or semiquantitative, per specimen, each single antibody stain procedure; manual</td>
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<tr>
<td>88361</td>
<td>Morphometric analysis, tumor immunohistochemistry (eg, Her-2/neu, estrogen receptor/progesterone receptor), quantitative or semiquantitative, per specimen, each single antibody stain procedure; using computer-assisted technology</td>
</tr>
<tr>
<td>0018U</td>
<td>Oncology (thyroid), microRNA profiling by RT-PCR of 10 microRNA sequences, utilizing fine needle aspirate, algorithm reported as a positive or negative result for moderate to high risk of malignancy</td>
</tr>
<tr>
<td>0022U</td>
<td>Targeted genomic sequence analysis panel, non-small cell lung neoplasia, DNA and RNA analysis, 23 genes, interrogation for sequence variants and rearrangements, reported as presence/absence of variants and associated therapy(ies) to consider</td>
</tr>
<tr>
<td>0023U</td>
<td>Oncology (acute myelogenous leukemia), DNA, genotyping of internal tandem duplication,</td>
</tr>
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</table>
p.D835, p.I836, using mononuclear cells, reported as detection or non-detection of FLT3 mutation and indication for or against the use of midostaurin

### HCPCS Codes

<table>
<thead>
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<th>HCPCS Codes</th>
<th>Description</th>
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<td>G0103</td>
<td>Prostate cancer screening; prostate specific antigen test (PSA)</td>
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### Considered Not Medically Necessary:

### CPT® Codes

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<th>CPT® Codes</th>
<th>Description</th>
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<tbody>
<tr>
<td>81327</td>
<td>SEPT9 (Septin9) (eg, colorectal cancer) methylation analysis</td>
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</table>

Considered Experimental/Investigational/Unproven when used to report any non-covered molecular testing for hematology-oncology indications outlined in the policy statements above:

### CPT® Codes

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<tr>
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<th>Description</th>
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<tbody>
<tr>
<td>81201</td>
<td>APC (adenomatous polyposis coli) (eg, familial adenomatosis polyposis [FAP], attenuated FAP) gene analysis; full gene sequence</td>
</tr>
<tr>
<td>81206</td>
<td>BCR/ABL1 (t(9;22)) (eg, chronic myelogenous leukemia) translocation analysis; major breakpoint, qualitative or quantitative</td>
</tr>
<tr>
<td>81209</td>
<td>BLM (Bloom syndrome, RecQ helicase-like) (eg, Bloom syndrome) gene analysis, 2281del6ins7 variant</td>
</tr>
<tr>
<td>81211</td>
<td>BRCA1, BRCA2 (breast cancer 1 and 2) (eg, hereditary breast and ovarian cancer) gene analysis; full sequence analysis and common duplication/deletion variants in BRCA1 (ie, exon 13 del 3.835kb, exon 13 dup 6kb, exon 14-20 del 26kb, exon 22 del 510bp, exon 8-9 del 7.1kb)</td>
</tr>
<tr>
<td>81229</td>
<td>Cytogenomic constitutional (genome-wide) microarray analysis; interrogation of genomic regions for copy number and single nucleotide polymorphism (SNP) variants for chromosomal abnormalities</td>
</tr>
<tr>
<td>81242</td>
<td>FANCC (Fanconi anemia, complementation group C) (eg, Fanconi anemia, type C) gene analysis, common variant (eg, IVS4+4A&gt;T)</td>
</tr>
<tr>
<td>81246</td>
<td>FLT3 (fms-related tyrosine kinase 3) (eg, acute myeloid leukemia), gene analysis; tyrosine kinase domain (TKD) variants (eg, D835, I836)</td>
</tr>
<tr>
<td>81261</td>
<td>IGH@ (Immunoglobulin heavy chain locus) (eg, leukemias and lymphomas, B-cell), gene rearrangement analysis to detect abnormal clonal population(s); amplified methodology (eg, polymerase chain reaction)</td>
</tr>
<tr>
<td>81264</td>
<td>IGK@ (Immunoglobulin kappa light chain locus) (eg, leukemia and lymphoma, B-cell), gene rearrangement analysis, evaluation to detect abnormal clonal population(s)</td>
</tr>
<tr>
<td>81275</td>
<td>KRAS (v-Ki-ras2 Kirsten rat sarcoma viral oncogene) (eg, carcinoma) gene analysis, variants in codons 12 and 13</td>
</tr>
<tr>
<td>81276</td>
<td>KRAS (Kirsten rat sarcoma viral oncogene homolog) (eg, carcinoma) gene analysis; additional variant(s) (eg, codon 61, codon 146)</td>
</tr>
<tr>
<td>81287</td>
<td>MGMT (0-6-methylguanine-DNA methyltransferase) (eg, glioblastoma multiforme), methylation analysis</td>
</tr>
<tr>
<td>81292</td>
<td>MLH1 (mutL homolog 1, colon cancer, nonpolyposis type 2) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; full sequence analysis</td>
</tr>
<tr>
<td>81295</td>
<td>MSH2 (mutS homolog 2, colon cancer, nonpolyposis type 1) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; full sequence analysis</td>
</tr>
<tr>
<td>81298</td>
<td>MSH6 (mutS homolog 6 [E. coli]) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; full sequence analysis</td>
</tr>
<tr>
<td>81310</td>
<td>NPM1 (nucleophosmin) (eg, acute myeloid leukemia) gene analysis, exon 12 variants</td>
</tr>
<tr>
<td>81311</td>
<td>NRAS (neuroblastoma RAS viral [v-ras] oncogene homolog) (eg, colorectal carcinoma), gene analysis, variants in exon 2 (eg, codons 12 and 13) and exon 3 (eg, codon 61)</td>
</tr>
<tr>
<td>CPT® Codes</td>
<td>Description</td>
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<tr>
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</tr>
<tr>
<td>81313</td>
<td>PCA3/KLK3 (prostate cancer antigen 3 [non-protein coding]/kallikrein-related peptidase 3 [prostate specific antigen]) ratio (eg, prostate cancer)</td>
</tr>
<tr>
<td>81315</td>
<td>PML/RARalpha, (t(15;17)), (promyelocytic leukemia/retinoic acid receptor alpha) (eg, promyelocytic leukemia) translocation analysis; common breakpoints (eg, intron 3 and intron 6), qualitative or quantitative</td>
</tr>
<tr>
<td>81321</td>
<td>PTEN (phosphatase and tensin homolog) (eg, Cowden syndrome, PTEN hamartoma tumor syndrome) gene analysis; full sequence analysis</td>
</tr>
<tr>
<td>81342</td>
<td>TRG@ (T cell antigen receptor, gamma) (eg, leukemia and lymphoma), gene rearrangement analysis, evaluation to detect abnormal clonal population(s)</td>
</tr>
<tr>
<td>81401</td>
<td>Molecular pathology procedure, Level 2 (eg, 2-10 SNPs, 1 methylated variant, or 1 somatic variant typically using nonsequencing target variant analysis, or detection of a dynamic mutation disorder/triplet repeat)</td>
</tr>
<tr>
<td>81479</td>
<td>Unlisted molecular pathology procedure</td>
</tr>
<tr>
<td>81500</td>
<td>Oncology (ovarian), biochemical assays of two proteins (CA-125 and HE-4), utilizing serum, with menopausal status, algorithm reported as a risk score</td>
</tr>
<tr>
<td>81503</td>
<td>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</td>
</tr>
<tr>
<td>81504</td>
<td>Oncology (tissue of origin), microarray gene expression profiling of &gt; 2000 genes, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as tissue similarity scores</td>
</tr>
<tr>
<td>81525</td>
<td>Oncology (colon), mRNA, gene expression profiling by real-time RT-PCR of 12 genes (7 content and 5 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a recurrence score</td>
</tr>
<tr>
<td>81540</td>
<td>Oncology (tumor of unknown origin), mRNA, gene expression profiling by real-time RT-PCR of 92 genes (87 content and 5 housekeeping) to classify tumor into main cancer type and subtype, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a probability of predicted main cancer type and subtype</td>
</tr>
<tr>
<td>81599</td>
<td>Unlisted multianalyte assay with algorithmic analysis</td>
</tr>
<tr>
<td>82387</td>
<td>Cathepsin-D</td>
</tr>
<tr>
<td>83520</td>
<td>Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise specified</td>
</tr>
<tr>
<td>83951</td>
<td>Oncoprotein; des-gama-carboxy-prothrombin (DCP)</td>
</tr>
<tr>
<td>84275</td>
<td>Sialic acid</td>
</tr>
<tr>
<td>84999</td>
<td>Unlisted chemistry procedure</td>
</tr>
<tr>
<td>88358</td>
<td>Morphometric analysis; tumor (eg, DNA ploidy)</td>
</tr>
<tr>
<td>0010M</td>
<td>Oncology (High-Grade Prostate Cancer), biochemical assay of four proteins (Total PSA, Free PSA, Intact PSA and human kallikrein 2 [hK2]) plus patient age, digital rectal examination status, and no history of positive prostate biopsy, utilizing plasma, prognostic algorithm reported as a probability score</td>
</tr>
<tr>
<td>0012M</td>
<td>Oncology (urothelial), mRNA, gene expression profiling by real-time quantitative PCR of five genes (MDK, HOXA13, CDC2 [CDK1], IGFBP5, and XCR2), utilizing urine, algorithm reported as a risk score for having urothelial carcinoma</td>
</tr>
<tr>
<td>0013M</td>
<td>Oncology (urothelial), mRNA, gene expression profiling by real-time quantitative PCR of five genes (MDK, HOXA13, CDC2 [CDK1], IGFBP5, and CXCR2), utilizing urine, algorithm reported as a risk score for having recurrent urothelial carcinoma</td>
</tr>
<tr>
<td>0019U</td>
<td>Oncology, RNA, gene expression by whole transcriptome sequencing, formalin-fixed paraffin embedded tissue or fresh frozen tissue, predictive algorithm reported as potential targets for therapeutic agents</td>
</tr>
</tbody>
</table>

**Tumor Profile/Gene Expression Classifier Testing**

Considered Medically Necessary when criteria in the applicable policy statements listed above are met:

<table>
<thead>
<tr>
<th>CPT® Codes</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPT® Codes</td>
<td>Description</td>
</tr>
<tr>
<td>------------</td>
<td>-------------</td>
</tr>
<tr>
<td>81235</td>
<td>EGFR (epidermal growth factor receptor) (eg, non-small cell lung cancer) gene analysis, common variants (eg, exon 19 LREA deletion, L858R, T790M, G719A, G719S, L861Q)</td>
</tr>
<tr>
<td>81519</td>
<td>Oncology (breast), mRNA, gene expression profiling by real-time RT-PCR of 21 genes, utilizing formalin-fixed paraffin embedded tissue, algorithm reported as recurrence score</td>
</tr>
<tr>
<td>81520</td>
<td>Oncology (breast), mRNA gene expression profiling by hybrid capture of 58 genes (50 content and 8 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a recurrence risk score</td>
</tr>
<tr>
<td>81521</td>
<td>Oncology (breast), mRNA, microarray gene expression profiling of 70 content genes and 465 housekeeping genes, utilizing fresh frozen or formalin-fixed paraffin-embedded tissue, algorithm reported as index related to risk of distant metastasis</td>
</tr>
<tr>
<td>81538</td>
<td>Oncology (lung), mass spectrometric 8-protein signature, including amyloid A, utilizing serum, prognostic and predictive algorithm reported as good versus poor overall survival</td>
</tr>
<tr>
<td>0008M</td>
<td>Oncology (breast), mRNA analysis of 58 genes using hybrid capture, on formalin-fixed paraffin-embedded (FFPE) tissue, prognostic algorithm reported as a risk score (Code deleted 12/31/2017)</td>
</tr>
</tbody>
</table>

**HCPCS Codes**

<table>
<thead>
<tr>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>S3854</td>
</tr>
</tbody>
</table>

**Considered Experimental/Investigational/Unproven:**

<table>
<thead>
<tr>
<th>CPT® Codes</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>86152</td>
<td>Cell enumeration using immunologic selection and identification in fluid specimen (eg, circulating tumor cells in blood);</td>
</tr>
<tr>
<td>86153</td>
<td>Cell enumeration using immunologic selection and identification in fluid specimen (eg, circulating tumor cells in blood); physician interpretation and report, when required</td>
</tr>
<tr>
<td>0009U</td>
<td>Oncology (breast cancer), ERBB2 (HER2) copy number by FISH, tumor cells from formalin fixed paraffin embedded tissue isolated using image-based dielectrophoresis (DEP) sorting, reported as ERBB2 gene amplified or non-amplified</td>
</tr>
<tr>
<td>0026U</td>
<td>Oncology (thyroid), DNA and mRNA of 112 genes, next-generation sequencing, fine needle aspirate of thyroid nodule, algorithmic analysis reported as a categorical result (“Positive, high probability of malignancy” or “Negative, low probability of malignancy”)</td>
</tr>
</tbody>
</table>

**Prostate Cancer Screening and Prognostic Tests**

Considered Medically Necessary when criteria in the applicable policy statements listed above are met:

<table>
<thead>
<tr>
<th>CPT® Codes</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>81313</td>
<td>PCA/KLK3 (prostate cancer antigen 3 [non-protein coding]/kallikrein-related peptidase 3 [prostate specific antigen]) ratio (eg, prostate cancer)</td>
</tr>
<tr>
<td>81479</td>
<td>Unlisted molecular pathology procedure</td>
</tr>
<tr>
<td>81539</td>
<td>Oncology (high-grade prostate cancer), biochemical assay of four proteins (Total PSA, Free PSA, Intact PSA and human kallikrein-2 [hK2]), utilizing plasma or serum, prognostic algorithm reported as a probability score</td>
</tr>
<tr>
<td>81551</td>
<td>Oncology (prostate), promoter methylation profiling by real-time PCR of 3 genes (GSTP1, APC, RASSF1), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a likelihood of prostate cancer detection on repeat biopsy (Code effective 01/01/2018)</td>
</tr>
<tr>
<td>84153</td>
<td>Oncology (prostate), promoter methylation profiling by real-time PCR of 3 genes (GSTP1, APC, RASSF1), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a likelihood of prostate cancer detection on repeat biopsy (Code effective 01/01/2018)Prostate specific antigen (PSA); total</td>
</tr>
<tr>
<td>84154</td>
<td>Prostate specific antigen (PSA); free</td>
</tr>
</tbody>
</table>
Immunoassay for tumor antigen, other antigen, quantitative (eg, CA 50, 72-4, 549), each

Considered Experimental/Investigational/Unproven:

<table>
<thead>
<tr>
<th>CPT® Codes</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0005U</td>
<td>Oncology (prostate) gene expression profile by real-time RT-PCR of 3 genes (ERG, PCA3, and SPDEF), urine, algorithm reported as risk score</td>
</tr>
<tr>
<td>0011M</td>
<td>Oncology, prostate cancer, mRNA expression assay of 12 genes (10 content and 2 housekeeping), RT-PCR test utilizing blood plasma and/or urine, algorithms to predict high-grade prostate cancer risk</td>
</tr>
</tbody>
</table>

Tumor Tissue-Based Molecular Assays for Prostate Cancer

Considered Medically Necessary when criteria in the applicable policy statements listed above are met:

<table>
<thead>
<tr>
<th>CPT® Codes</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>81479†</td>
<td>Unlisted molecular pathology procedure</td>
</tr>
<tr>
<td>81541</td>
<td>Oncology (prostate), mRNA expression profiling by real-time RT-PCR of 46 genes (31 content and 15 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a disease-specific mortality risk score</td>
</tr>
<tr>
<td>81599††</td>
<td>Unlisted multianalyte assay with algorithmic analysis</td>
</tr>
</tbody>
</table>

†Note: Considered Medically Necessary when used to report Decipher® Prostate Cancer Classifier Assay or ProMark Proteomic Prognostic Test

††Note: Considered Medically Necessary when used to report OncotypeDx Genomic Prostate Score

Hematologic Cancer and Myeloproliferative and Myelodysplastic Disease

Considered Medically Necessary when criteria in the applicable policy statements listed above are met:

<table>
<thead>
<tr>
<th>CPT® Codes</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>81170</td>
<td>ABL1 (ABL proto-oncogene 1, non-receptor tyrosine kinase) (eg, acquired imatinib tyrosine kinase inhibitor resistance), gene analysis, variants in the kinase domain</td>
</tr>
<tr>
<td>81175</td>
<td>ASXL1 (additional sex combs like 1, transcriptional regulator) (eg, myelodysplastic syndrome, myeloproliferative neoplasms, chronic myelomonocytic leukemia), gene analysis; full gene sequence</td>
</tr>
<tr>
<td>81176</td>
<td>ASXL1 (additional sex combs like 1, transcriptional regulator) (eg, myelodysplastic syndrome, myeloproliferative neoplasms, chronic myelomonocytic leukemia), gene analysis; targeted sequence analysis (eg, exon 12)</td>
</tr>
<tr>
<td>81208</td>
<td>BCR/ABL1 (t(9;22)) (eg, chronic myelogenous leukemia) translocation analysis; other breakpoint, qualitative or quantitative</td>
</tr>
<tr>
<td>81219</td>
<td>CALR (calreticulin) (eg, myeloproliferative disorders), gene analysis, common variants in exon 9</td>
</tr>
<tr>
<td>81334</td>
<td>RUNX1 (runt related transcription factor 1) (eg, acute myeloid leukemia, familial platelet disorder with associated myeloid malignancy), gene analysis, targeted sequence analysis (eg, exons 3-8)</td>
</tr>
<tr>
<td>81270</td>
<td>JAK2 (Janus kinase 2) (eg, myeloproliferative disorder) gene analysis, p.Val617Phe (V617F) variant</td>
</tr>
<tr>
<td>81401</td>
<td>Molecular pathology procedure, Level 2 (eg, 2-10 SNPs, 1 methylated variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat)</td>
</tr>
<tr>
<td>Code</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>81402</td>
<td>Molecular pathology procedure, Level 3 (eg, &gt;10 SNPs, 2-10 methylated variants, or 2-10 somatic variants [typically using non-sequencing target variant analysis], immunoglobulin and T-cell receptor gene rearrangements, duplication/deletion variants of 1 exon, loss of heterozygosity [LOH], uniparental disomy [UPD])</td>
</tr>
<tr>
<td>81403</td>
<td>Molecular pathology procedure, Level 4 (eg, analysis of single exon by DNA sequence analysis, analysis of &gt;10 amplicons using multiplex PCR in 2 or more independent reactions, mutation scanning or duplication/deletion variants of 2-5 exons)</td>
</tr>
<tr>
<td>81479</td>
<td>Unlisted molecular pathology procedure</td>
</tr>
<tr>
<td>0017U</td>
<td>Oncology (hematolymphoid neoplasia), JAK2 mutation, DNA, PCR amplification of exons 12-14 and sequence analysis, blood or bone marrow, report of JAK2 mutation not detected or detected</td>
</tr>
<tr>
<td>0027U</td>
<td>JAK2 (Janus kinase 2) (eg, myeloproliferative disorder) gene analysis, targeted sequence analysis exons 12-15</td>
</tr>
</tbody>
</table>

**Considered Experimental/Investigational/Unproven:**

<table>
<thead>
<tr>
<th>CPT® Codes</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0016U</td>
<td>Oncology (hematolymphoid neoplasia), RNA, BCR/ABL1 major and minor breakpoint fusion transcripts, quantitative PCR amplification, blood or bone marrow, report of fusion not detected or detected with quantitation</td>
</tr>
</tbody>
</table>

**Occult Neoplasms**

**Considered Medically Necessary when criteria in the applicable policy statements listed above are met:**

<table>
<thead>
<tr>
<th>CPT® Codes</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>83520</td>
<td>Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise specified</td>
</tr>
<tr>
<td>84181</td>
<td>Protein; Western Blot, with interpretation and report, blood or other body fluid</td>
</tr>
<tr>
<td>84182</td>
<td>Protein; Western Blot, with interpretation and report, blood or other body fluid, immunological probe for band identification, each</td>
</tr>
<tr>
<td>86255</td>
<td>Fluorescent noninfectious agent antibody; screen, each antibody</td>
</tr>
<tr>
<td>86256</td>
<td>Fluorescent noninfectious agent antibody; titer, each antibody</td>
</tr>
</tbody>
</table>

**Solid Tumor Cancers**

**Considered Experimental/Investigational/Unproven when used to report any non-covered tumor analysis or gene expression profiling outlined in the policy statements above:**

<table>
<thead>
<tr>
<th>CPT® Codes</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>81479</td>
<td>Unlisted molecular pathology procedure</td>
</tr>
<tr>
<td>84999</td>
<td>Unlisted chemistry test</td>
</tr>
<tr>
<td>88299</td>
<td>Unlisted cytogenetic study</td>
</tr>
<tr>
<td>88399</td>
<td>Unlisted surgical pathology procedure</td>
</tr>
<tr>
<td>89240</td>
<td>Unlisted miscellaneous pathology test</td>
</tr>
<tr>
<td>0003U</td>
<td>Oncology (ovarian) biochemical assays of five proteins (apolipoprotein A-1, CA 125 II, follicle stimulating hormone, human epididymis protein 4, transferring), utilizing serum algorithm reported as a likelihood score</td>
</tr>
</tbody>
</table>

**Other Tumor Profile Testing**

**Considered Experimental/Investigational/Unproven when used to report topographic genotyping:**
<table>
<thead>
<tr>
<th>CPT® Codes</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>81479</td>
<td>Unlisted molecular pathology procedure</td>
</tr>
<tr>
<td>84999</td>
<td>Unlisted chemistry procedure</td>
</tr>
</tbody>
</table>


**References**


American Thyroid Association (ATA) Guidelines Taskforce on Thyroid Nodules and Differentiated Thyroid Cancer. THYROID. Volume 19, Number 11, 2009


87. Filipits M, Nielsen TO, Rudas M, Greil R, Stöger H, Jakesz R, et al. The PAM50 risk-of-recurrence score predicts risk for late distant recurrence after endocrine therapy in postmenopausal women with...


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