Policy

*Please see amendment for Pennsylvania Medicaid at the end of this CPB.

I. Aetna considers any of the following serum tumor markers for the stated indication medically necessary:

A. Prostate-specific antigen (PSA) for prostate cancer screening (see CPB 0521 - Prostate Cancer Screening), staging, monitoring response to therapy, and detecting disease recurrence.

B. Carcinoembryonic antigen (CEA) for any of the following:

1. As a preoperative prognostic indicator in members with known colorectal carcinoma or mucinous appendiceal carcinoma when it will assist in staging and surgical treatment planning; or
2. Pancreatic cyst fluid CEA for distinguishing mucinous from non-mucinous malignant pancreatic cysts; or
3. To detect asymptomatic recurrence of colorectal cancer after surgical and/or medical treatment for the diagnosis of colorectal cancer (not as a screening test for colorectal cancer); or
4. To monitor response to treatment for metastatic colorectal cancer.
C. CEA for cholangiocarcinoma, gallbladder cancer, lung cancer, medullary thyroid cancer, metastatic breast cancer, mucinous ovarian cancer, and occult primary.

D. CEA for evaluation of jaundice, abnormal liver function tests (LFTs) or for obstruction/abnormality on liver imaging.

E. 1p19q codeletion molecular cytogenetic analysis for astrocytomas and gliomas

F. 5-hydroxyindoleacetic acid (5-HIAA) for neuroendocrine tumors

G. ALK gene fusion as a molecular biomarker in non-small cell lung cancer

H. ALK gene rearrangement for diffuse large B cell lymphoma, peripheral T-cell lymphoma, and post-transplant lymphoproliferative disorder

I. ALK translocations for selecting candidates for crizotinib (Xalkori) in inflammatory myofibroblastic tumor

J. APC for familial adenomatous polyposis when criteria are met in CPB 0140 - Genetic Testing; and for desmoid fibromatosis; experimental for other indications.

K. Afirma Thyroid FNA analysis for assessing fine needle aspiration samples from thyroid nodules that are indeterminate; experimental for other indications. Repeat testing is considered experimental and investigational.

L. Alpha fetoprotein (AFP) for the following indications:
   hepatocellular carcinoma; mediastinal mass; ovarian cancer; pelvic mass; testicular cancer; testicular mass; thymic carcinoma; and thymoma.

M. Alfa fetoprotein (AFP) for testing for hepatocellular carcinoma in hepatitis B carriers, or for persons with cirrhosis and one or more of the following risk factors: alcohol use; alpha-1 antitrypsin deficiency; Asian female at least 50 years of age; Asian male at least 40 years of age; family history of HCC; genetic hemochromatosis; hepatitis C; nonalcoholic steatohepatitis; and stage 4 primary biliary cirrhosis.

N. Alpha fetoprotein (AFP): serial measurements to diagnose germ cell tumors in members with adenocarcinoma, or carcinoma not otherwise specified, involving mediastinal nodes; or the diagnosis and monitoring of hepatocellular
carcinoma (e.g., before considering liver transplantation).

O. BCR/ABL fluorescent in situ hybridization (FISH) for lymphoblastic lymphoma, acute myeloid leukemia, acute lymphocytic leukemia and chronic myelogenous leukemia; experimental for other indications.

P. Beta-2 microglobulin (B2M) for multiple myeloma, non-Hodgkin's lymphoma and Waldenström's macroglobulinemia/ lymphoplasmacytic lymphoma.

Q. BRAF V600 mutation for indeterminate thyroid nodules, hairy cell leukemia; gastrointestinal stromal tumors; Lynch syndrome testing for persons meeting criteria in CPB 140 - Genetic Testing; melanoma for vemurafenib, dabrafenib, and trametinib (see CPB 0715 - Pharmacogenomic and Pharmacodynamic Testing); and colorectal cancer if KRAS nonmutated; experimental for other indications.

R. Breast Cancer Index to assess necessity of adjuvant chemotherapy in females or males with recently diagnosed breast tumors, where all of the following criteria are met:

1. Breast cancer is nonmetastatic (node negative); and
2. Breast tumor is estrogen receptor positive; and
3. Breast tumor is HER2 receptor negative; and
4. Adjuvant chemotherapy is not precluded due to any other factor (e.g., advanced age and/or significant co-morbidities); and
5. Member and physician (prior to testing) have discussed the potential results of the test and agree to use the results to guide therapy.

S. Cancer antigen 125 (CA 125) levels for any of the following:

1. As a preoperative diagnostic aid in women with ovarian masses that are suspected to be malignant, such that arrangements can be made for intraoperative availability of a gynecological oncologist if the CA 125 is increased; or
2. As a screening test for ovarian cancer when there is a family history of hereditary ovarian cancer syndrome (a
pattern of clusters of ovarian cancer within two or more generations), where testing is performed concurrently with transvaginal ultrasound and prophylactic salpingooophorectomy has not been performed. For this indication, screening is considered medically necessary every six months beginning at 30 years of age or 10 years before the earliest age of the first diagnosis of ovarian cancer in the family; or

3. Diagnosis of ovarian cancer in women with new symptoms (bloating, pelvic or abdominal pain, difficulty eating or feeling full quickly, or urinary frequency and urgency) that have persisted for three or more weeks, where the clinician has performed a pelvic and rectal examination and suspects ovarian cancer; or

4. In members with adenocarcinoma of unknown primary, to rule out ovarian cancer; or

5. In members with known ovarian cancer, as an aid in the monitoring of disease, response to treatment, detection of recurrent disease, or assessing value of performing second-look surgery.

T. CA 15-3: Serial measurements of CA 15-3 (also known as CA 27-29 or Truquant RIA) in following the course of treatment in women diagnosed with breast cancer, especially advanced metastatic breast cancer (an increasing CA 15-3 level may suggest treatment failure).

U. CA 19-9 to monitor the clinical response to therapy or detect early recurrence of disease in members with known gastric cancer, pancreatic cancer, gallbladder cancer, cholangiocarcinoma or adenocarcinoma of the ampulla of Vater.

V. CA 19-9 to rule out cholangiocarcinoma in persons with primary sclerosing cholangitis undergoing liver transplantation.

W. CA 19-9 for evaluation of jaundice, abnormal liver function tests (LFTs) or obstruction/abnormality on imaging.

X. CA 19-9 as a tumor marker for mucinous appendiceal carcinoma.

Y. CALCA (calcitonin) expression for medullary thyroid cancer
or for adenocarcinoma or anaplastic/undifferentiated tumors of the head and neck.

Z. CALB2 (calretinin) expression for lung cancer and occult primary.

AA. CD 20, for determining eligibility for anti-CD20 treatment (rituximab) -- see CPB 0314 - **Rituximab (Rituxan)**.

AB. CD 25, for determining eligibility for denileukin diftitox (Ontak) treatment.

AC. CD 31 immunostaining, for diagnosis of angiosarcoma.

AD. CD 33, for determining eligibility for anti-CD33 (gemtuzumab, Mylotarg) treatment.

AE. CD 52, for determining eligibility for anti-CD52 (alemtuzumab, Campath) treatment.

AF. CD117 (c-kit), for determining eligibility for treatment with imatinib mesylate (Gleevec).

AG. CHGA (Chromogranin A) expression for neuroendocrine tumors, non-small cell lung cancer, Merkel cell carcinoma and occult primary.

AH. Cyclin D1, for diagnosis and predicting disease recurrence of mantle cell lymphoma.

AI. DecisionDx-UM (Castle Biosciences, Phoenix, AZ) for risk stratification of persons with localized uveal melanoma.

AJ. EndoPredict (also known as 12-gene score) to assess necessity of adjuvant chemotherapy in females or males with recently diagnosed breast tumors, where all of the following criteria are met:

1. Breast cancer is nonmetastatic (node negative); and
2. Breast tumor is estrogen receptor positive; and
3. Breast tumor is HER2 receptor negative; and
4. Adjuvant chemotherapy is not precluded due to any other factor (e.g., advanced age and/or significant comorbidities); and
5. Member and physician (prior to testing) have discussed the potential results of the test and agree to use the results to guide therapy.

AK. Epidermal growth factor receptor (EGFR) mutation testing
for predicting response to EGFR-targeting tyrosine kinase inhibitors (erlotinib (Tarceva), gefitinib (Iressa), afatinib (Gilotrif)) in non-small cell lung cancer.

AL. FLT3 gene mutation testing for acute myeloid leukemia (AML).

AM. Human chorionic gonadotropin (HCG), serial measurement to diagnose germ cell tumors in members with adenocarcinoma, or carcinoma not otherwise specified, involving mediastinal nodes, or to monitor treatment in members with known trophoblastic tumors (invasive hydatidiform moles and choriocarcinomas) and germ cell tumors (teratocarcinoma and embryonal cell carcinoma) of the ovaries or testes, or to monitor for relapse after remission is achieved.

AN. Beta human chorionic gonadotropin (beta-HCG) for mediastinal mass; ovarian cancer; pelvic mass; testicular mass; testicular cancer; thymoma; or thymic carcinoma.

AO. Human epidermal growth factor receptor 2 (HER2) evaluation in breast, gastric and esophageal cancer - see CPB 0313 - Trastuzumab (Herceptin), Ado-Trastuzumab (Kadcyla) and Pertuzumab (Perjeta).

AP. IGH@ (Immunoglobulin heavy chain locus), gene rearrangement analysis to detect abnormal clonal population(s) in non-Hodgkin’s lymphomas, hairy cell leukemia, and post-transplant lymphoproliferative disorder.

AQ. IGK@ (Immunoglobulin kappa light chain locus), gene rearrangement analysis, evaluation to detect abnormal clonal population(s) for non-Hodgkin’s lymphoma, systemic light chain amyloidosis

AR. Isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) gene mutation for AML, chondrosarcomas, or gliomas and glioblastomas

AS. INHA (inhibin) expression for ovarian cancer or pelvic mass

AT. Lactate dehydrogenase (LDH) for acute lymphoblastic leukemia (ALL), bone cancer, kidney cancer, kidney mass, lung cancer, multiple myeloma, non-Hodgkin’s lymphoma, pelvic mass, ovarian cancer, testicular cancer, or testicular mass.

AU. K-ras (KRAS) mutation analysis, with BRAF reflex testing, to
predict non-response to cetuximab (Erbitux) and panitumumab (Vectibix) in the treatment of anal adenocarcinoma, metastatic colorectal cancer and small bowel adenocarcinoma; K-ras (KRAS) mutation analysis to predict non-response to erlotinib (Tarceva) in the treatment of non-small cell lung cancer; experimental for all other indications.

AV. Mammaprint to assess necessity of adjuvant chemotherapy in females or males with recently diagnosed breast tumors, where all of the following criteria are met:

1. Breast cancer is nonmetastatic (node negative) or with 1-3 involved ipsilateral axillary lymph nodes; and
2. Breast tumor is estrogen receptor positive or progesterone receptor positive; and
3. Breast tumor is HER2 receptor negative (Rationale: adjuvant chemotherapy with trastuzumab (Herceptin) is considered to be medically necessary regardless of Mammaprint score for HER2 receptor positive lesions); and
4. Member is determined to be at "high clinical risk" of recurrence using Adjuvant! Online (www.adjuvantonline.com) (see page 20 of MINDACT study supplement for definitions of high clinical risk: http://www.nejm.org/doi/suppl/10.1056/NEJMoa1602253/suppl_file/nejmoa1602253_appendix.pdf); and
5. Adjuvant chemotherapy is not precluded due to any other factor (e.g., advanced age and/or significant co-morbidities); and
6. Member and physician (prior to testing) have discussed the potential results of the test and agree to use the results to guide therapy.

AW. Measurement of estrogen and progesterone receptors on primary breast cancers, and on metastatic lesions if the results would influence treatment planning.

AX. MLH1, MSH2, MSH6 for persons meeting HNPCC/Lynch
Syndrome testing criteria in **CPB 0140 - Genetic Testing**; colorectal cancer in persons under age 50; and all persons with Stage II colon cancer; experimental for all other indications,

AY. Mycosis fungoides, diagnosis: polymerase chain reaction (PCR) for T-cell receptor gamma chain gene rearrangement as an adjunct to the histopathologic diagnosis of mycosis fungoides.

AZ. Myeloperoxidase (MPO) immunostaining, FLT3-ITD, CEBPA mutation, NPM1 mutation, and KIT mutation for diagnosis of acute myeloid leukemia.

BA. NPM1 in acute myeloid leukemia (AML); experimental for other indications.

BB. PAM50 Risk of Recurrence (ROR) Score (also known as Prosigna Breast Cancer Prognostic Gene Signature Assay)\(^2\) to assess necessity of adjuvant chemotherapy in females or males with recently diagnosed breast tumors, where all of the following criteria are met:

1. Breast cancer is nonmetastatic (node negative); and
2. Breast tumor is estrogen receptor positive; and
3. Breast tumor is HER2 receptor negative; and
4. Adjuvant chemotherapy is not precluded due to any other factor (e.g., advanced age and/or significant co-morbidities); and
5. Member and physician (prior to testing) have discussed the potential results of the test and agree to use the results to guide therapy.

BC. PDGFRA for gastrointestinal stromal tumors (GIST).

BD. PDGFRB testing for myelodysplastic syndromes (MDS) and dermatofibrosarcoma protuberans.

BE. PML/RARA for acute promyelocytic leukemia; experimental for all other indications.

BF. PTEN for persons meeting Cowden syndrome testing criteria in **CPB 0140 - Genetic Testing**; experimental for all other indications.

BG. Placental alkaline phosphatase (PLAP), to diagnose germ cell seminoma and non-seminoma germ cell tumors in
unknown primary cancers.

BH. Quest Diagnostics Thyroid Cancer Mutation Panel for assessing fine needle aspiration samples from thyroid nodules that are indeterminate; experimental for other indications. Repeat testing is considered experimental and investigational.

BI. ROS-1 to predict response to crizotinib (Xalkori) for the treatment of non-small cell lung cancer (NSCLC).

BJ. RUNX1 for myelodysplastic syndrome.

BK. Steroid hormone receptor status in both pre-menopausal and post-menopausal members to identify individuals most likely to benefit from endocrine forms of adjuvant therapy and therapy for recurrent or metastatic breast cancer.

BL. Targeted hematologic genomic sequencing panel (5-50 genes) for myelodysplastic syndromes.

BM. Targeted solid organ genomic sequencing panel (5-50 genes) for non-small cell lung cancer.

BN. T-cell receptor gene rearrangements (TRA@, TRB@, TRD@, TRG@) for T-cell prolymphocytic leukemia, T-cell large granular lymphocytic leukemia, nasal type extranodal NK/T-cell lymphoma, peripheral T-cell lymphoma, primary cutaneous CD30+ T-cell lymphoproliferative disorders, Castleman's disease, mycosis fungoides/Sezary syndrome

BO. ThyGenX (formerly Mirinform Thyroid) for assessing fine needle aspiration samples from thyroid nodules that are indeterminate; experimental for other indications. Repeat testing is considered experimental and investigational.

BP. ThyraMIR as a reflex test following ThyGenX for assessing fine needle aspiration samples from thyroid nodules that are indeterminate; experimental for other indications. Repeat testing is considered experimental and investigational.

BQ. Thymidine kinase for chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL)

BR. Thyroglobulin antibodies for thyroid cancer

BS. Thyroglobulin (TG) expression for thyroid cancer, occult primary, and adenocarcinoma or anaplastic/undiifferentiated tumors of the head and neck

BT. Thyroid transcription factor-1 (TTF-1) for lung cancer or neuroendocrine tumors
BU. Thyroseq for assessing fine needle aspiration samples from thyroid nodules that are indeterminate; experimental for other indications. Repeat testing is considered experimental and investigational.

BV. Oncotype Dx (also known as 21 gene RT-PCR test) to assess necessity of adjuvant chemotherapy in females or males with recently diagnosed breast tumors, where all of the following criteria are met:

1. Breast cancer is nonmetastatic (node negative) or with 1-3 involved ipsilateral axillary lymph nodes; and
2. Breast tumor is estrogen receptor positive; and
3. Breast tumor is HER2 receptor negative or breast tumor is HER2 receptor positive and less than 1 cm in diameter. (Rationale: adjuvant chemotherapy with trastuzumab (Herceptin) is considered to be medically necessary regardless of an Oncotype Dx score for HER2 receptor positive lesions 1 cm or more in diameter); and
4. Adjuvant chemotherapy is not precluded due to any other factor (e.g., advanced age and/or significant co-morbidities); and
5. Member and physician (prior to testing) have discussed the potential results of the test and agree to use the results to guide therapy (i.e., member will forgo adjuvant chemotherapy if Oncotype Dx score is low).

BW. Urokinase plasminogen activator (uPA) and plasminogen activator inhibitor 1 (PAI-1) to assess necessity of adjuvant chemotherapy in females or males with recently diagnosed breast tumors, where all of the following criteria are met:

1. Breast cancer is nonmetastatic (node negative); and
2. Breast tumor is estrogen receptor positive; and
3. Breast tumor is HER2 receptor negative; and
4. Adjuvant chemotherapy is not precluded due to any other factor (e.g., advanced age and/or significant co-morbidities); and
5. Member and physician (prior to testing) have discussed the potential results of the test and agree to use the
results to guide therapy.

In addition, urokinase plasminogen activator (uPA) and plasminogen activator inhibitor 1 (PAI-1) is considered medically necessary for the determination of prognosis in patients with newly diagnosed, node negative breast cancer.

BX. Veristrat proteomic testing for patients with advanced NSCLC, whose tumors were without EGFR and anaplastic lymphoma kinase (ALK) mutations, who had progressed after at least one chemotherapy regimen, and for whom erlotinib was considered an appropriate treatment.

BY. WT-1 gene expression for non-small cell lung cancer and occult primary

BZ. ZAP-70, for assessing prognosis and need for aggressive therapy in persons with chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL).

1 Either standard node dissection negative by hematoxylin and eosin (H&E) staining or sentinel node negative by H&E staining (if sentinel node is negative by H&E, but immunoassay is positive, then still considered node negative for this purpose). In addition, women with isolated tumor cells in lymph nodes (micrometastases) are considered node negative.

More than one Oncotype Dx test may be medically necessary for persons with breast cancer who have two or more histologically distinct tumors that meet medical necessity criteria. Repeat Oncotype Dx testing or testing of multiple tumor sites in the same person has no proven value for other indications. Oncotype Dx is considered experimental and investigational for ductal carcinoma in situ (OncotypeDx DCIS), colon cancer (OncotypeDx Colon), prostate cancer (OncotypeDx Prostate) and all other indications.

2 Aetna considers use of more than one type of test to determine necessity of adjuvant therapy in breast cancer (Oncotype Dx Breast, Breast Cancer Index, EndoPredict,
PAM50, Mammmaprint, or uPA and PAI-1) experimental and investigational.

II. Aetna considers urinary biomarkers (e.g., bladder tumor antigen (BTA) (e.g., BTA Stat and BTA TRAK), nuclear matrix protein (NMP22) test, the fibrin/fibrinogen degradation products (Aura-Tek FDfP) test, or fluorescence in situ hybridization (FISH) (e.g., UroVysion Bladder Cancer test medically necessary in any of the following conditions:

A. Follow-up of treatment for bladder cancer; or
B. Monitoring for eradication of bladder cancer; or
C. Recurrences after eradication.

Aetna considers the BTA Stat test, the NMP22 test, the Aura-Tek FDP test, or the UroVysion fluorescent in situ hybridization (FISH) test experimental and investigational for screening of bladder cancer, evaluation of hematuria, and diagnosing bladder cancer in symptomatic individuals, and all other indications.

III. Aetna considers the use of fluorescence immunocytology (e.g., ImmunoCyt/uCyt) medically necessary as an adjunct to cystoscopy or cytology in the monitoring of persons with bladder cancer.

Aetna considers the ImmunoCyte/uCyt immunohistochemistry test experimental and investigational in the evaluation of hematuria, diagnosing bladder cancer, or for screening for bladder cancer in asymptomatic persons.

IV. Aetna considers genetic testing for Janus Kinase 2 (JAK2) mutations in persons with chronic myeloproliferative disorders (CMPDs) medically necessary for the following indications: 1) qualitative assessment of JAK2-V617F sequence variant using methods with detection thresholds of up to 5% for initial diagnostic assessment of adult patients presenting with symptoms of CMPD; 2) diagnostic assessment of polycythemia vera in adults; and 3) differential diagnosis of essential thrombocytosis and primary myelofibrosis from
reactive conditions in adults.

Aetna considers genetic testing for Janus Kinase 2 (JAK2) mutations in persons with chronic myeloproliferative disorders (CMPDs) experimental and investigational for any other indication including: 1) diagnostic assessment of myeloproliferative disorders in children; 2) quantitative assessment of JAK2-V617F allele burden subsequent to qualitative detection of JAK2-V617F.

V. Aetna considers each of the following experimental and investigational. The peer reviewed medical literature does not support these tests as having sufficient sensitivity or specificity necessary to define their clinical role:

1. CEA used for all other indications including any of the following:

   a. As a screening test for colorectal cancer; or
   
   b. As a sole determinant to treat a colorectal cancer
      member with adjuvant therapy or systemic therapy
      for presumed metastatic disease; or
   
   c. For diagnosis of esophageal carcinoma; or
   
   d. For screening, diagnosis, staging or routine
      surveillance of gastric cancer.

2. AFP for the diagnosis of trophoblastic tumors and oncologic indications other than those listed above.

3. Assaying for loss of heterozygosity (LOH) on the long arm of chromosome 18 (18q) or deleted in colon cancer (DCC) protein (18q-LOH/DCC) for colorectal cancer

4. Biomarker Translation (BT) test for breast cancer and other indications

5. BioSpeciFx, including Comprehensive Tumor Profiling for any indication

6. BRAF mutation analysis in lung cancer

7. Breast Cancer Gene Expression Ratio (HOXB13:IL17BR)

8. CA 125 for all other indications including use as a screening test for colorectal cancer or ovarian cancer
(other than as indicated above) or for differential diagnosis of members with symptoms of colonic disease
9. CA 19-9 for all other indications including breast, colorectal, esophageal, gastro-esophageal, liver, or uterine cancer; pancreatic pseudocyst; screening persons with primary sclerosing cholangitis without signs or symptoms of cholangiocarcinoma; or screening persons with primary sclerosing cholangitis for development of cholangiocarcinoma.
10. Carcinoembryonic antigen cell adhesion molecule 6 (CEACAM6) (e.g., Benign Diagnostics Risk Test) for breast atypical hyperplasia and for predicting the risk of breast cancer.
11. Carcinoembryonic antigen cellular adhesion molecule-7 (CEACAM-7) expression as a predictive marker for rectal cancer recurrence
12. Caris Target Now Molecular Profiling Test
13. CDX2 as a prognostic biomarker for colon cancer
14. CEA, Cyfra21-1 (a cytokeratin 19 fragment), p53, squamous cell carcinoma antigen (SCC-Ag) and vascular endothelial growth factor C (VEGF-C) for diagnosis of esophageal carcinoma
15. Circulating cell-free nucleic acids in colorectal cancer
16. Circulating tumor cell (CTC) assays (e.g., CellSearch assay) for all indications, including, but not limited to metastatic breast, colorectal, melanoma, and prostate cancers
17. CK5, CK14, p63, and Racemase P504S testing for prostate cancer
18. c-Met expression for predicting prognosis in persons with advanced NSCLC and colorectal cancer, and other indications
19. Cofilin (CFL1) as a prognostic and drug resistance marker in non-small cell lung cancer
20. ColonSentry test for screening of colorectal cancer
21. ColoPrint, CIMP, LINE-1 hypomethylation, and Immune cells for colon cancer
22. Colorectal Cancer DSA (Almac Diagnostics, Craigavon, UK)
23. ConfirmMDx for prostate cancer
24. CxBladder test for bladder cancer
25. Cyclin D1 and FADD (Fas-associated protein with death domain) for head and neck squamous cell carcinoma
26. Decipher test (a RNA biomarkers assay) for prostate cancer
27. Decision DX-Melanoma (Castle Biosciences, Phoenix, AZ)
28. DCIS Recurrence Score
29. Des-gamma-carboxy prothrombin (DCP) (also known as "prothrombin produced by vitamin K absence or antagonism II" [PIVKA II]) for diagnosing and monitoring hepatocellular carcinoma (HCC) and other indications
30. EarlyCDT-Lung test
31. EGFR gene expression analysis for transitional (urothelial) cell cancer
32. EGFRVIII for glioblastoma multiforme
33. EML4-ALK as a diagnostic tool for stage IV non-small-cell lung cancer
34. Estrogen and progesterone receptors when used alone to assign a member with breast cancer to prognostic groupings since they are relatively weak predictors of long-term relapse and breast cancer related mortality rates
35. Excision repair cross-complementation group 1 protein (ERCC1) for persons with NSCLC, colon or with gastric cancer who are being considered for treatment with platinum-based chemotherapy, and other indications
36. ExoDx Prostate (IntelliScore)
37. Fibrin/fibrinogen degradation products (FDP) test (e.g., DR-70 or Onko-Sure) for colorectal cancer
38. FoundationOne and FoundationOne Heme
39. Galectin-3 for prostate cancer
40. Gene hypermethylation for prostate cancer
41. GeneKey (GeneKey Corp., Boston, MA)
42. GeneSearch Breast Lymph Node (BLN) assay
43. Glutathione-S-transferase P1 (GSTP1) for screening, detection and management of prostate cancer
44. Guanylyl cyclase c (GCC or GUCY2C) (e.g., Previstage GCC Colorectal Cancer State Test) for colorectal cancer
45. Guardant360
46. HER2 testing of appendiceal cancer
47. HERmark testing for breast cancer and other indications
48. HMGB1 and RAGE in cutaneous malignancy (e.g., basal cell carcinoma, melanoma, and squamous cell carcinoma)
49. Human epididymis protein 4 (HE4) (e.g., Elecsys HE4 assay) for endometrial cancer, ovarian cancer, or evaluation of pelvic mass, including to assist in the determination of referral for surgery to a gynecologic oncologist or general surgery, and for other indications
50. IHC4 (e.g., NexCourse IHC4 by AQUA Technology) for breast cancer
51. Insight DX Breast Cancer Profile
52. Ki67 for breast cancer
53. Ki-67 in upper tract urinary carcinoma
54. 4Kscore
55. Lectin-reactive alpha-fetoprotein (AFP-L3) for liver cancer
56. Liquid biopsy (e.g., CancerIntercept, GeneStrat, FoundationACT) for any indication, including, but not limited to, breast cancer, colorectal cancer, lung cancer, melanoma, or ovarian cancer.
57. Long non-coding RNA in gallbladder cancer
58. Mammastrat
59. Mass spectrometry-based proteomic profiling (e.g., Xpresys Lung) for indeterminate pulmonary nodules
60. MatePair targeted rearrangements (whole genome next-generation sequencing) for hematolymphoid neoplasia and solid organ neoplasia
61. Microarray-based gene expression profile testing using the MyPRS test for multiple myeloma
62. Micro-RNAs (miRNAs) miRview mets and miRview mets2 (Rosetta Genomics Laboratories, Philadelphia, PA; Rosetta Genomics Ltd., Rehovot, Israel)
63. MLH1 tumor promoter hypermethylation for endometrial cancer
64. Molecular Intelligence Services, including MI Profile and MI Profile PLUS (formerly Target Now Molecular Profiling Test, including Target Now Select and Target Now Comprehensive)
65. Molecular subtyping profile (e.g., BluePrint) for breast cancer
66. MUC1 in gastric cancer
67. Mucin 4 expression as a predictor of survival in colorectal cancer
68. Mucin 5AC (MUC5AC) as serum marker for biliary tract cancer
69. My Prognostic Risk Signature (MyPRS) (Signal Genetics LLC, New York, NY)
70. NRAS mutation for selecting persons with metastatic colorectal cancer who may benefit from anti-VEGF antibody bevacizumab; to predict disease prognosis and select persons with melanoma who may benefit from tyrosine kinase inhibitor therapies, and other indications
71. OncoVantage
72. OVA1 test
73. OvaCheck test
74. OvaSure
75. OncInsights (Intervention Insights, Grand Rapids, MI)
76. p16 protein expression as a prognostic marker in non-oropharyngeal squamous cell carcinoma (cancer of the oral cavity, hypopharynx, or larynx)
77. Pathwork Tissue of Origin test
78. Perceptua Bronchial Genomic Classifier
79. Phosphatidylinositol-4,5-bisphosphonate 3-kinase, catalytic subunit alpha polypeptide gene (PIK3CA) for predicting disease prognosis and selecting individuals with metastatic colorectal cancer who are being considered for treatment with EGFR antagonists cetuximab and panitumumab, and other indications
80. PreOvar test for the KRAS-variant to determine ovarian cancer risk
81. ProLaris for prostate cancer
82. ProOnc TumorSourceDx test (Prometheus Laboratories, San Diego, CA) to identify tissue or origin for metastatic tumor
83. Prostate core mitotic test
84. Prostate Px and Post-Op Px for predicting recurrence of prostate cancer
85. Proveri prostate cancer assay (PPCA)
86. PSA for screening women with breast cancer or for differentiating benign from malignant breast masses
87. PTEN gene expression for non-small cell lung cancer
88. Ras oncogenes (except KRAS and BRAF)
89. ResponseDx Colon
90. Ribonucleotide reductase subunit M1 (RRM1) for persons with NSCLC who are being considered for treatment with gemcitabine-based chemotherapy, and other indications
91. ROMA (Risk of Ovarian Malignancy Algorithm) for ovarian cancer
92. Rotterdam Signature 76-gene panel
93. SelectMDx for prostate cancer
94. Serum amyloid A as a biomarker for endometrioid endometrioid carcinoma to monitor disease recurrence and target response to therapy
95. TargetPrint gene expression test for evaluation of estrogen receptor, progesterone receptor, and HER2 receptor status in breast cancer
96. The 41-gene signature assay
97. Theros CancerType ID (bioTheranostics Inc., San Diego, CA)
98. Thymidylate synthase
99. TMPRSS fusion genes for prostate cancer
100. Topographic genotyping (Pancragen (formerly PathFinderTG))
101. Total (whole) gene sequencing for cancer
102. TP53 mutation analysis for ovarian cancer
103. UroCor cytology panels (DD23 and P53) for bladder cancer
104. Vascular Endothelial Growth Factor (VEGF)
105. Vascular endothelial growth factor receptor 2 (VEGFR2) expression for identifying persons with colorectal cancer that is likely to respond to VEGF inhibition, and other indications
106. Any of the following circulating tumor markers also is considered experimental and investigational for screening asymptomatic subjects for cancer, diagnosis, staging, routine surveillance of cancer and monitoring the response to treatment:
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<tr>
<th>Code</th>
<th>Name</th>
<th>Description</th>
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<td>Topoisomerase II Alpha (TOP2A)</td>
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Background

A tumor marker is a substance such as a protein, antigen or hormone in the body that may indicate the presence of cancer. Generally, these markers are specific to certain types of cancer and can be detected in blood, urine and tissue samples. The body may produce the marker in response to cancer or the tumor itself may produce the marker. The detection of tumor markers may be used to determine a diagnosis or as an indicator of disease (cancer) progression. It can also be used to document clinical response to treatment. Tumor markers include, but may not be limited to, alpha-fetoprotein (AFP), CA 15-3/CA 27.29, CA 19-9, CA-125, carcinoembryonic antigen (CEA) and prostate-specific antigen (PSA).

Tumor markers are normally produced in low quantities by cells in the body. Detection of a higher-than-normal serum level by radioimmunoassay or immunohistochemical techniques usually indicates the presence of a certain type of cancer. Currently, the main use of tumor markers is to assess a cancer’s response to treatment and to check for recurrence. In some types of cancer, tumor marker levels may reflect the extent or stage of the disease and can be useful in predicting how well the disease will respond to treatment. A decrease or return to normal in the level of a tumor marker may indicate that the cancer has responded favorably to therapy. If the tumor marker level rises, it may
indicate that the cancer is spreading. Finally, measurements of tumor marker levels may be used after treatment has ended as a part of follow-up care to check for recurrence.

However, in many cases the literature states that measurements of tumor marker levels alone are insufficient to diagnose cancer for the following reasons: (1) tumor marker levels can be elevated in people with benign conditions; (2) tumor marker levels are not elevated in every person with cancer, especially in the early stages of the disease; and (3) many tumor markers are not specific to a particular type of cancer; and (4) the level of a tumor marker can be elevated by more than one type of cancer.

Examples of tumor markers include:

- **5-Hydroxyindoleacetic acid (5-HIAA)** -- the main metabolite of serotonin, used as a marker in the evaluation of carcinoid tumors;
- **Beta-2-Microglobulin (B2M)** – A protein found on the surface of many cells. High levels of B2M are an indicator of certain kinds of cancer, including chronic lymphocytic leukemia, non-Hodgkin's lymphoma and multiple myeloma or kidney disease;
- **Beta Human Chorionic Gonadotropin (beta HCG)** – A type of tumor marker that may be found in higher than normal amounts in individuals with some types of cancer;
- **Calcitonin** – Hormone secreted by the thyroid that lowers blood calcium;
- **Calretinin** – A calcium-binding protein that is used as a marker in the evaluation of lung cancer and other diseases.
- **Chromogranin A** – A protein found inside neuroendocrine cells, which releases chromogranin A and other hormones into the blood. Chromogranin A may be found in higher than normal amounts in individuals with certain neuroendocrine tumors, small cell lung cancer, prostate cancer and other conditions.
- **Guanylyl cyclase c (GCC)** – An enzyme that may be expressed only in the cells that line the intestine from the duodenum to the rectum.
- **Inhibin** – One of two hormones (designated inhibin-A and inhibin-B) secreted by the gonads (by Sertoli cells in the male
and the granulosa cells in the female) and inhibits the production of follicle-stimulating hormone (FSH) by the pituitary gland;

- Lactate Dehydrogenase (LDH) – Marker used to monitor treatment of testicular cancer;
- Mucin-1 (MUC-1) – Carbohydrate antigen elevated in individuals with tumors of the breast, ovary, lung and prostate as well as other disorders;
- Napsin A – Protein used as a marker in the evaluation of lung cancer;
- Prealbumin – Marker of nutritional status and a sensitive indicator of protein synthesis. Also referred to as transthyretin;
- Prostate Specific Antigen (PSA) – Substance produced by the prostate gland. Levels of PSA in the blood often increase in men with prostate cancer.
- Thyroglobulin – Protein found in the thyroid gland. Some thyroglobulin can be found in the blood and this amount may be measured after thyroid surgery to determine whether thyroid cancer has recurred;
- Thyroid Transcription Factor-1 (TTF-1) – A protein that is used as a tumor marker in the evaluation of lung cancer;
- Transferrin – A protein in blood plasma that carries iron derived from food intake to the liver, spleen and bone marrow.

Tumors may be evaluated with histology, which involves examination of the structure, especially the microscopic structure, of organic tissues. Methods of detecting tumor markers include, but are not limited to: Fluorescence in Situ Hybridization (FISH) – Laboratory technique used to detect small deletions or rearrangements in chromosomes. Immunohistochemical (IHC) Analysis – Laboratory process of detecting an organism in tissues with antibodies.

Gene mutation testing can purportedly be used to find somatic mutations in cancerous cells that are not inherited. Some examples of genes that may have somatic mutations include: IDH1 and IDH2 genes (associated with acute myeloid leukemia [AML], gliomas and chondrosarcomas); NPM1 and FLT3 genes (associated with AML).
Individualized molecular tumor profiling is a laboratory method of testing a panel of tumor markers, which may include genetic as well as biochemical markers, to establish a personalized molecular profile of a tumor to recommend treatment options.

Mass spectrometry based proteomic profiling (eg, Veristrat, Xpresys Lung) is a multivariate serum protein test that uses mass spectrometry and proprietary algorithms to analyze proteins in an individual’s serum.

Next-generation sequence (NGS) tests use select genes to purportedly identify molecular growth drivers for improved risk stratification and targeted therapies. Examples include: FoundationOne and OncoVantage for solid tumor cancers; FoundationOne Heme for hematological cancers and sarcomas; and ThyGenX for indeterminate thyroid nodules.

Liquid biopsy refers to serum testing for DNA fragments that are shed by cancer cells and released into the bloodstream. This method is purportedly used for screening, diagnosis and/or monitoring of cancer cells that may otherwise require a tissue sample.

Multianalyte assays with algorithmic analyses (MAAAs) are laboratory measurements that use a mathematical formula to analyze multiple markers that may be associated with a particular disease state and are designed to evaluate disease activity or an individual’s risk for disease. The laboratory performs an algorithmic analysis using the results of the assays and sometimes other information, such as sex and age and converts the information into a numeric score, which is conveyed on a laboratory report. Generally, MAAAs are exclusive to a single laboratory which owns the algorithm. MAAAs have been proposed for the evaluation of pelvic masses, including assisting in the determination of referral for surgery to a gynecologic oncologist or to a general surgeon.

Topographic genotyping (eg, PathFinderTG) is a test that examines a panel of 15 to 20 genetic markers in tissue biopsy or other tissue specimens to purportedly aid in the determination of
PSA

Prostate Specific Antigen (PSA) is a substance produced by the prostate gland. Levels of PSA in the blood often increase in men with prostate cancer. Elevated levels of Prostate-Specific Antigen (PSA) may also be found in the blood of men with benign prostate conditions, such as prostatitis and benign prostatic hyperplasia (BPH). While PSA does not allow distinction between benign prostate conditions and cancer, an elevated PSA level may indicate that other tests are necessary to determine whether cancer is present. PSA levels have been shown to be useful in monitoring the effectiveness of prostate cancer treatment, and in checking for recurrence after treatment has ended. Use of PSA for screening remains very controversial. Although researchers are in the process of studying the value of PSA along with digital rectal exams for routine screening of men ages 55 to 74 for prostate cancer; and the literature does not show at this time whether using PSA to screen for prostate cancer actually does reduce the number of deaths caused by this cancer. The American Cancer Society recommends clinicians and patients consider screening with PSA and digital rectal exam for African American men and men with familial tendency age 40 or older and all men age 50 or older.

Cancer Care Ontario guidelines on active surveillance of prostate cancer (Morash, et al., 2015) state that the active surveillance protocol should include the following tests: PSA test every 3 to 6 months; digital rectal examination every year, and a 12- to 14-core confirmatory transrectal ultrasound (TRUS) biopsy (including anterior directed cores) within 6 to 12 months, then serial biopsy a minimum of every 3 to 5 years thereafter. The guidelines state that "[c]urrent evidence shows that PSA kinetics does not reliably predict disease stability or reclassification to higher risk state. There was conflicting evidence whether PSA is a good predictor of disease progression or reclassification. Differences were also found in the ability of different measures of PSA, such as PSA velocity, PSA density, and PSA doubling time for predicting progression or reclassification. PSA monitoring is considered a
necessary component of an AS protocol, but a rising PSA may be best viewed as a trigger for reappraisal (e.g., MRI, repeat biopsy) rather than a trigger for intervention."

PCA3

Prostate cancer antigen 3 (PCA3, also known as DD3) is a gene that has been found to be highly overexpressed in prostate cancer. This gene has been investigated as a potential diagnostic marker for prostate cancer. However, there are no published clinical outcome studies of the effectiveness of the PCA3 gene in screening, diagnosis or management of prostate cancer.

Prostate cancer antigen 3 (PCA3) (Progensa, Gene-Probe, Inc.) encodes a prostate-specific mRNA. It is one of the most prostate cancer-specific genes identified, with over-expression in about 95% of cancers tested. The PCA3 urine assay is an amplified nucleic acid assay, which uses transcription-mediated amplification (TMA) to quantify PCA3 and PSA mRNA in prostate cells found in urine samples. The PCA3 score is calculated as the ratio between PCA3 and PSA mRNA. The main target population of this non-invasive test is men with raised PSA but a negative prostate biopsy. Other target groups include men with a slightly raised PSA, as well as men with signs and symptoms suggestive of prostate cancer.

van Gils and colleagues (2007) stated that PCA3 is a promising prostate cancer marker. These investigators performed a multi-center study to validate the diagnostic performance of the PCA3 urine test established in an earlier single-institution study. The first voided urine after digital rectal examination (DRE) was collected from a total of 583 men with serum PSA levels between 3 and 15 ng/ml who were to undergo prostate biopsies. These researchers determined the PCA3 score in these samples and correlated the results with the results of the prostate biopsies. A total of 534 men (92 %) had an informative sample. The area under the receiver-operating characteristic curve, a measure of the diagnostic accuracy of a test, was 0.66 for the PCA3 urine test and 0.57 for serum PSA. The sensitivity for the PCA3 urine test was 65 %, the specificity was 66 % (versus 47 % for serum PSA),
and the negative predictive value was 80%. The authors concluded that the findings of this multi-center study validated the diagnostic performance of the PCA3 urine test in the largest group studied thus far using a PCA3 gene-based test.

Marks and associates (2007) examined the potential utility of the investigational PCA3 urine assay to predict the repeat biopsy outcome. Urine was collected after DRE (3 strokes per lobe) from 233 men with serum PSA levels persistently 2.5 ng/ml or greater and at least one previous negative biopsy. The PCA3 scores were determined using a highly sensitive quantitative assay with TMA. The ability of the PCA3 score to predict the biopsy outcome was assessed and compared with the serum PSA levels. The RNA yield was adequate for analysis in the urine samples from 226 of 233 men (i.e., the informative specimen rate was 97%). Repeat biopsy revealed prostate cancer in 60 (27%) of the 226 remaining subjects. Receiver operating characteristic curve analysis yielded an area under the curve of 0.68 for the PCA3 score. In contrast, the area under the curve for serum PSA was 0.52. Using a PCA3 score cutoff of 35, the assay sensitivity was 58% and specificity 72%, with an odds ratio of 3.6. At PCA3 scores of less than 5, only 12% of men had prostate cancer on repeat biopsy; at PCA3 scores of greater than 100, the risk of positive biopsy was 50%. The authors concluded that in men undergoing repeat prostate biopsy to rule out cancer, the urinary PCA3 score was superior to serum PSA determination for predicting the biopsy outcome. The high specificity and informative rate suggest that the PCA3 assay could have an important role in prostate cancer diagnosis.

Groskopf et al (2007) reported that the PCA3 score is independent of prostate volume and was highly correlated with the risk of positive biopsy. The PCA3 test was performed on 529 men scheduled for prostate biopsy. Overall, the PCA3 score had a sensitivity of 54% and a specificity of 74%. A PCA3 score of less than 5 was associated with a 14% risk of positive biopsy, while a PCA3 score of greater than 100 was associated with a 69% risk of positive biopsy.

Haese et al (2007) presented preliminary results from a European multicenter study of PCA3. Enrolled patients had a PSA level of
less than or equal to 2.5 ng/mL, had 1 or 2 previous negative biopsies, and were scheduled for repeat biopsy. The specificity of the PCA3 score (cutoff 35) was found to be 78%, and the sensitivity was 67%. Patients with a PCA3 score of greater than or equal to 35 had a 33% probability of a positive repeat biopsy, compared to a 6% probability for those with a PCA3 score of less than 35.

In a review on biomarkers for prostate cancer detection, Parekh, et al. (2007) stated that prostate stem cell antigen, alpha-methyl coenzyme-A racemase, PCA3, early prostate cancer antigen, hepsin and human kallikrein 2 are promising markers that are currently undergoing validation.

An assessment by the BlueCross BlueShield Association Technology Evaluation Center (BCBSA, 2008) found that, in general, PCA3 assay results to date are preliminary; interpretation of results has not been standardized and clinical utility studies of decision-making for initial biopsy, repeat biopsy or treatment have not been reported.

Tosoian et al (2010) evaluated the relationship between PCA3 and prostate biopsy results in men in a surveillance program. Urine specimens were obtained from 294 men with prostate cancer enrolled in the Johns Hopkins surveillance program. The follow-up protocol included semi-annual free and total PSA measurements, digital rectal examination and annual surveillance prostate biopsy. Cox proportional hazards regression was used to evaluate the association between PCA3 results and progression on surveillance biopsy (defined as Gleason pattern 4 or 5, more than 2 positive biopsy cores or more than 50% involvement of any core with cancer). Patients with progression on biopsy (12.9%) had a mean PCA3 score similar to that of those without progression (60.0 versus 50.8, p = 0.131). Receiver operating characteristics analysis suggested that PCA3 alone could not be used to identify men with progression on biopsy (area under the curve = 0.589, 95% CI 0.496 to 0.683, p = 0.076). After adjustment for age and date of diagnosis PCA3 was not significantly associated with progression on biopsy (p = 0.15). The authors concluded that in men with low risk prostate cancer who were carefully selected for surveillance
the PCA3 score was not significantly associated with short-term biopsy progression. They stated that further analysis is necessary to assess the usefulness of PCA3 in combination with other biomarkers or in selected subsets of patients undergoing surveillance.

While there are studies examining the positive and negative predictive values of the PCA3 urine assay, there is currently a lack of evidence of the effect of this test on management of individuals with or suspected of prostate cancer. The PCA3 urine assay shows promise as a prostate cancer diagnostic tool, however, more research is needed to ascertain the clinical value of this assay for screening and diagnostic purposes.

An assessment of PCA3 prepared for the Agency for Healthcare Research and Quality (2013) concluded: "For diagnostic accuracy, there was a low strength of evidence that PCA3 had better diagnostic accuracy for positive biopsy results than tPSA elevations, but insufficient evidence that this led to improved intermediate or long-term health outcomes. For all other settings, comparators, and outcomes, there was insufficient evidence."

The Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group (2013) found insufficient evidence to recommend prostate cancer antigen 3 (PCA3) testing to inform decisions for when to rebiopsy previously biopsy-negative patients for prostate cancer or to inform decisions to conduct initial biopsies for prostate cancer in at-risk men (e.g., previous elevated prostate-specific antigen test or suspicious digital rectal examination). The EGAPP Working Group found insufficient evidence to recommend PCA3 testing in men with cancer-positive biopsies to determine if the disease is indolent or aggressive in order to develop an optimal treatment plan. The EGAPP Working Group concluded that, based on the available evidence, the overall certainty of clinical validity to predict the diagnosis of prostate cancer using PCA3 is deemed "low." The EGAPP Working Group discouraged clinical use for diagnosis unless further evidence supports improved clinical validity. The EGAPP Working Group also found that, based on the available evidence, the overall certainty of net health benefit is deemed
“low.” The EGAPP Working Group discourages clinical use unless further evidence supports improved clinical outcomes.

Guidelines from the European Association of Urology (2015) state that "[b]iological markers, include urine markers such as PCA3, the TMPRSS2: ERG fusion gene or PSA isoforms such as the Phi index, appear promising as does genomics on the tissue sample itself. However, further study data will be needed before such markers can be used in standard clinical practice."

A Cancer Care Ontario Guideline on prostate cancer surveillance (Morash, et al., 2015), which has been endorsed by the American Society for Clinical Oncology (2016), did not include PCA3 level in their recommendation because evidence of PCA3 to predict disease reclassification in prostate cancer was lacking.

National Institute for Health and Care Excellence (NICE)’s clinical practice guideline on “Diagnosing prostate cancer: PROGENSA PCA3 assay and Prostate Health Index” (2015) stated that “The PROGENSA PCA3 assay and the Prostate Health Index are not recommended for use in people having investigations for suspected prostate cancer, who have had a negative or inconclusive transrectal ultrasound prostate biopsy”. The assessment cited studies finding that adding the PCA3 score to clinical assessment and MRI had very little effect on the size of the reported area under the curve, with minimal change in derived sensitivity and specificity for clinical assessment with MRI compared with clinical assessment using MRI and the PCA3 assay.

In a Lancet review of prostate cancer, Attard, et al. (2016) stated that "[s]everal studies have so far proven inconclusive as to whether PCA3 is useful to selectively detect aggressive prostate cancers."

B15

Hutchinson et al (2005) stated that in tissue-based assays, thymosin beta15 (B15) has been shown to correlate with prostate cancer and with recurrence of malignancy. To be clinically effective, it must be shown that thymosin B15 is released by the
tumor into body fluids in detectable concentrations. These researchers developed a quantitative assay that can measure clinically relevant levels of thymosin B15 in human urine. Sixteen antibodies were raised against recombinant thymosin B15 and/or peptide conjugates. One antibody, having stable characteristics over the wide range of pH and salt concentrations found in urine and minimal cross-reactivity with other beta thymosins, was used to develop a competitive enzyme-linked immunosorbent assay (ELISA). Urinary thymosin B15 concentration was determined for control groups; normal (n = 52), prostate intraepithelial neoplasia (PIN, n = 36), and patients with prostate cancer; untreated (n = 7) with subsequent biochemical failure, radiation therapy (n = 17) at risk of biochemical recurrence. The operating range of the competition ELISA fell between 2.5 and 625 ng/ml. Recoveries exceeded 75%, and the intra- and inter-assay coefficients of variability were 3.3% and 12.9%, respectively. No cross-reactivity with other urine proteins was observed. A stable thymosin B15 signal was recovered from urine specimens stored at -20 degrees C for up to 1 year. At a threshold of 40 (ng/dl)/microg protein/mg creatinine), the assay had a sensitivity of 58% and a specificity of 94%. Relative to the control groups, thymosin B15 levels were greater than this threshold in a significant fraction of patients with prostate cancer (p < 0.001), including 5 of the 7 patients who later experienced PSA recurrence. The authors concluded that an ELISA that is able to detect thymosin B15 at clinically relevant concentrations in urine from patients with prostate cancer has been established. They noted that the assay will provide a tool for future clinical studies to validate urinary thymosin B15 as a predictive marker for recurrent prostate cancer.

CEA

Carcinoembryonic antigen (CEA) is a normal cell product that is over-expressed by adenocarcinomas, primarily of the colon, rectum, breast, and lung. It is normally found in small amounts in the blood of most healthy people, but may become elevated in people who have cancer or some benign conditions.

CEA is an oncofetal glycoprotein present in the gastrointestinal tract and body fluids of the embryo and fetus (Chin, et al., 2006).
It is also present in certain adult gastrointestinal cells, including the mucosal cells of the colorectum, and small amounts are present in blood. Blood levels are often elevated in patients with disseminated cancers and in some patients with nonmalignant disease.

According to the available literature, the primary use of CEA is in monitoring colorectal cancer, especially when the disease has metastasized. CEA is also used after treatment to check for recurrence of colorectal cancer. However, the literature indicates a wide variety of other cancers can produce elevated levels of this tumor marker, including melanoma; lymphoma; and cancers of the breast, lung, pancreas, stomach, cervix, bladder, kidney, thyroid, liver, and ovary. Elevated CEA levels can also occur in patients with non-cancerous conditions, including inflammatory bowel disease, pancreatitis, and liver disease.

The American Society of Clinical Oncology (ASCO)'s update of recommendations for the use of tumor markers in gastrointestinal cancer (Gershon, et al., 2006) stated that post-operative CEA levels should be performed every 3 months for stage II and III disease for at least 3 years if the patient is a potential candidate for surgery or chemotherapy of metastatic disease.

CA-125

Cancer antigen 125 (CA-125) is a test that evaluates ovarian cancer treatment. CA-125 is a protein that is found more in ovarian cancer cells than in other cells. CA-125 is expressed by >80 percent of non-mucinous ovarian epithelial neoplasms (Chin et al, 2006). Approximately half of women with metastatic ovarian cancer have an elevated CA-125 level.

The Gynecologic Cancer Foundation, the Society of Gynecologic Oncologists, and the American Cancer Society have issued a consensus statement to promote early detection of ovarian cancer, which recommends that women who have symptoms, including bloating, pelvic or abdominal pain, difficulty eating or feeling full quickly, and urinary frequency and urgency, are urged
to see a gynecologist if symptoms are new and persist for more than three weeks (ACS, 2007; SGO, 2007). Ovarian cancer is among the deadliest types of cancer because diagnosis usually comes very late, after the cancer has spread. If the cancer is found and surgically removed before it spreads outside the ovary, the five year survival rate is 93%. However, only 19% of cases are detected early enough for that kind of successful intervention. It is estimated that 22,430 new cases and 15,280 deaths will be reported in 2007 (ACS, 2007). The consensus statement recommendations are based on studies that show the above symptoms appeared in women with ovarian cancer more than in other women (Goff, et al., 2004; Daly & Ozols, 2004). The recommendations acknowledge that there is not consensus on what physicians should do when patients present with these symptoms. According to a consensus statement issued by the Gynecologic Cancer Foundation, pelvic and rectal examination in women with the symptoms is one first step. If there is a suspicion of cancer, the next step may be a transvaginal ultrasound to check the ovaries for abnormal growths, enlargement, or telltale pockets of fluid that can indicate cancer. Testing for CA-125 levels should also be considered.

There is no evidence available that measurement of CA-125 can be effectively used for widespread screening to reduce mortality from ovarian cancer, nor that the use of this test would result in decreased rather than increased morbidity and mortality. According to the available literature, not all women with elevated CA 125 levels have ovarian cancer. CA 125 levels may also be elevated by cancers of the uterus, cervix, pancreas, liver, colon, breast, lung, and digestive tract. Non-cancerous conditions that can cause elevated CA 125 levels include endometriosis, pelvic inflammatory disease, peritonitis, pancreatitis, liver disease, and any condition that inflames the pleura. Menstruation and pregnancy can also cause an increase in CA 125. However, according to the available literature, changes in CA 125 levels can be effectively used in the management of treatment for ovarian cancer. In women with ovarian cancer being treated with chemotherapy, the literature suggests a falling CA 125 level generally indicates that the cancer is responding to treatment and increased survival is expected. Increasing CA 125 levels during or
after treatment, on the other hand, may suggest that the cancer is not responding to therapy or that residual cancer remains. According to the available literature, failure of the CA 125 level to return to normal after three cycles of chemotherapy indicates residual tumor, early treatment failure and decreased survival. Under accepted guidelines, CA 125 levels can also be used to monitor patients for recurrence of ovarian cancer. Although an elevated CA 125 level is highly correlated with the presence of ovarian cancer, the literature suggests a normal value does not exclude residual or recurrent disease.

Aetna's preventive services guidelines are based on the recommendations of leading primary care medical professional organizations and federal public health agencies. None of these organizations recommend routine screening of average-risk, asymptomatic women with serum CA-125 levels for ovarian cancer. These organizations have concluded that serum CA-125 levels are not sufficiently sensitive or specific for use as a screening test for ovarian cancer, and that the harms of such screening outweigh the benefits.

The American College of Obstetricians and Gynecologists (2002) has stated that "[u]nfortunately, there is no screening test for ovarian cancer that has proved effective in screening low-risk asymptomatic women. Measurement of CA 125 levels and completion of pelvic ultrasonography (both abdominal and transvaginal) have been the two tests most thoroughly evaluated.... Data suggest that currently available tests do not appear to be beneficial for screening low-risk, asymptomatic women because their sensitivity, specificity, positive predictive value, and negative predictive value have all been modest at best. Because of the low incidence of disease, reported to be approximately one case per 2,500 women per year, it has been estimated that a test with even 100% sensitivity and 99% specificity would have a positive predictive value of only 4.8%, meaning 20 of 21 women undergoing surgery would not have primary ovarian cancer. Unfortunately, no test available approaches this level of sensitivity or specificity."

The National Cancer Institute (2004) has stated: "There is
insufficient evidence to establish that screening for ovarian cancer with serum markers such as CA 125 levels, transvaginal ultrasound, or pelvic examinations would result in a decrease in mortality from ovarian cancer. A serious potential harm is the false-positive test result, which may lead to anxiety and invasive diagnostic procedures. There is good evidence that screening for ovarian cancer with the tests above would result in more diagnostic laparoscopies and laparotomies than new ovarian cancers found. Unnecessary oophorectomies may also result."

The U.S. Preventive Services Task Force (2004) recommends against routine screening with serum CA-125 level for ovarian cancer. The Task Force concluded that the potential harms of such screening outweigh the potential benefits.

**HE4**

Human Epididymis Protein 4 (HE4) is a secreted glycoprotein that is being studied as a potential marker for ovarian cancer.

A variety of other tumor markers have been investigated for early detection of ovarian cancer as well as different combinations of tumor markers complementary to CA 125 that could potentially offer greater sensitivity and specificity than CA 125 alone. Preliminary studies on HE4 (human epididymis protein 4), a marker for ovarian cancer, reported similar sensitivity to CA 125 when comparing ovarian cancer cases to healthy controls, and a higher sensitivity when comparing ovarian cancer cases to benign gynecologic disease (Hellstrom, et al., 2003 & 2008; Moore, et al., 2008;) However, an assessment on genomic tests for ovarian cancer prepared by Duke University for the Agency for Healthcare Research and Quality (AHRQ, 2006) stated, "Although research remains promising, adaptation of genomic tests into clinical practice must await appropriately designed and powered studies in relevant clinical settings." Further studies are needed to determine if HE4 significantly adds to the sensitivity of CA 125 while maintaining a high specificity.

NCCN guidelines (2016) state that data show that HE4 and several other markers do not increase early enough to be useful in
detecting early-stage ovarian cancer.

**CA 15-3**

Cancer antigen 15-3 (CA 15-3) is a serum cancer antigen that has been used in the management of patients with breast cancer. According to the available literature, its low detection rate in early stage disease indicates that CA 15-3 cannot be used to screen or diagnose patients with breast cancer. It has been widely used to monitor the effectiveness of treatment for metastatic cancer. Elevated serum CA 15-3 concentrations are found in 5 percent of stage I, 29 percent of stage II, 32 percent of stage III and 95 percent of stage IV carcinoma of the breast (Chin, et al, 2006). Most (96 percent) patients with a CA 15-3 increase of greater than 25 percent have disease progression. Most (nearly 100 percent) patients with a CA 15-3 decrease of greater than 50 percent are responding to treatment.

Cancers of the ovary, lung, and prostate may also raise CA 15-3 levels. The literature indicates elevated levels of CA 15-3 may be associated with non-cancerous conditions, such as benign breast or ovarian disease, endometriosis, pelvic inflammatory disease, and hepatitis.

Similar to the CA 15-3 antigen, CA 27-29 is found in the blood of most breast cancer patients. The literature indicates CA 27-29 levels may be used in conjunction with other procedures (such as mammograms and measurements of other tumor marker levels) to check for recurrence in women previously treated for stage II and stage III breast cancer. CA 27-29 levels can also be elevated by cancers of the colon, stomach, kidney, lung, ovary, pancreas, uterus, and liver. First trimester pregnancy, endometriosis, ovarian cysts, benign breast disease, kidney disease, and liver disease are non-cancerous conditions that can also elevate CA 27-29 levels.

Elevated CA 27.29 levels are primarily associated with metastatic breast cancer, where it can be used to monitor the course of disease, response to treatment, and detect disease recurrence (Chin, et al., 2006). Elevated serum CA 27.29 concentrations are
found in 95 percent of stage IV breast cancer. In addition, CA 27.29 has been found to be elevated in lung (43 percent), pancreas (47 percent), ovarian (56 percent), and liver (55 percent) cancer.

**CA 19-9**

Cancer antigen 19-9 (CA 19-9) is a mucin-glycoprotein first identified from a human colorectal carcinoma cell line and is present in epithelial tissue of the stomach, gall bladder, pancreas and prostate (Chin, et al., 2006). Concentrations are increased in patients with pancreatic, gastric, and colon cancer as well as in some nonmalignant conditions. Increasing levels generally indicate disease progression, whereas decreasing levels suggest therapeutic response.

Initially found in colorectal cancer patients, CA 19-9 has also been identified in patients with pancreatic, stomach, hepatocellular cancer, and bile duct cancer. In those who have pancreatic cancer, the literature indicates higher levels of CA 19-9 tend to be associated with more advanced disease. Although the sensitivity of the CA 19-9 level in patients with pancreatic cancer is relatively high, the specificity is lowered by elevations that occur in patients with benign pancreatic or liver disease. Non-cancerous conditions that may elevate CA 19-9 levels include gallstones, pancreatitis, cirrhosis of the liver, and cholecystitis. Although excellent correlations have been reported between CA 19-9 measurements and relapse in patients with pancreatic cancer who are followed after surgical resection, no patient has been salvaged by the earlier diagnosis of relapse, a fact that reflects the lack of effective therapy.

Guidelines from the National Comprehensive Cancer Network (NCCN, 2010) state that measurement of CA 19-9 should be considered in evaluating patients with intrahepatic or extrahepatic cholangiocarcinoma and gallbladder cancer. The guidelines note that CA 19-9 is often elevated in persons with cholangiocarcinoma or gallbladder cancer, although this marker is not specific for these cancers. Nehls, et al. (2004) considered CA19-9 as one of the several new potential tumor markers for the
diagnosis of cholangiocarcinoma. Levy, et al. (2005) aimed to characterize the test properties of CA 19-9 and of a change in CA 19-9 over time in predicting cholangiocarcinoma in patients with primary sclerosing cholangitis. Charts of 208 patients were reviewed. Fourteen patients had cholangiocarcinoma. Median CA 19-9 was higher with cholangiocarcinoma (15 versus 290 U/ml, p < 0.0001). A cutoff of 129 U/ml provided: sensitivity 78.6%, specificity 98.5%, adjusted positive predictive value 56.6% and negative predictive value 99.4%. The median change over time was 664 U/ml in cholangiocarcinoma compared to 6.7 U/ml in primary sclerosing cholangitis alone (p < 0.0001). A cutoff of 63.2 U/ml for change in CA 19-9 provided: sensitivity 90%, specificity 98% and positive predictive value 42%.

CA 19-9 is produced by adenocarcinomas of the pancreas, stomach, gall-bladder, colon, ovary, and lung, and it is shed into the circulation. Although numerous studies have addressed the potential utility of CA 19-9 in adenocarcinoma of the colon and rectum, the sensitivity of CA 19-9 was always less than that of the CEA test for all stages of disease. Its use for screening asymptomatic populations has been hampered by a false-positive rate of 15% to 30% in patients with non-neoplastic diseases of the pancreas, liver, and biliary tract. Only a few studies have addressed the use of CA 19-9 in monitoring patients' post-primary therapy. Significant postsurgical decreases are observed for CA 19-9, but these decreases have not been correlated with survival or disease-free interval. In monitoring response to treatment, decreases in CEA have been found to more accurately reflect response to therapy than did decreases of CA 19-9. Progressive increases of the marker may signal disease progression in 25% of the patients who express the CA 19-9 marker, but this monitoring provides only a minimal lead time of 1 to 3 months. Monitoring with CA 19-9 has not been shown to improve the management of patients with colorectal cancer. The serum CA 19-9 level does not add significant information to that provided by CEA, which is currently regarded as the marker of choice for this neoplasm.

Sinakos and colleagues (2011) evaluated the long-term outcomes in Mayo Clinic patients presenting with primary sclerosing
cholangitis (PSC) between 2000 and 2010 (n= 73) for incidence of cholangiocarcinoma (CCA). The results showed initial levels of CA 19-9 in patients without CCA were significantly lower than those from patients with CCA (p < 0.0001). No factors known to affect CA 19-9 levels were identified in 33% of the patients without CCA; endoscopic treatment and recurrent bacterial cholangitis were associated with levels of CA 19-9 in 26% and 22% of these patients, respectively.

Juntermanns (2011) prospectively analyzed a bile duct tumor database and retrieved records of 238 patients who underwent surgery between 1999 and 2008. Their findings included that pre-operative CA19-9 serum levels did not show a statically reliable differentiation between benign or malignant dignity. The authors concluded that current diagnostics cannot differentiate malignant from benign tumor masses in the hepatic hilum with required reliability. The authors further concluded that administration of CIK cells, thymus factor, IL-2 and IFN-alpha after AHSCT could improve the immunologic function of patients, and TH1/TH2 ratio may virtually reflect the immune status of patients, but that more information is required to make prognostic assessments of immune reconstruction and the long-term survival rate.

Sarbia et al (1993) investigated 69 adenocarcinomas of the esophagogastric junction and found high rates of antigen expression were found for the “intestinal” markers CA 19-9 (between 55.5% and 100%) and BW 494 (between 42.9 and 86.7%). The authors concluded that these data, in combination with CK-20 expression, PGII, and 2B5 indicate that the distribution of adenocarcinomas with gastric and/or intestinal differentiation at the esophagogastric junction forms a continuum with out clear-cut borders. This study has not been replicated and NCCN guidelines for Esophageal and Esophagogastric Junction Cancers does not include recommendations for CA 19-9 testing for these indications (NCCN, 2011).

The American Society of Clinical Oncology (ASCO)’s update of recommendations for the use of tumor markers in gastrointestinal cancer (Gershon, et al., 2006) stated that for
pancreatic cancer, CA 19-9 can be measured every 1 to 3 months for patients with locally advanced or metastatic disease receiving active therapy.

Mucinous carcinoma of the appendix is a rare entity most commonly associated with primary tumors of the appendix and colon, and for which spread is generally confined to the abdominal cavity (Andreopoulou et al, 2007). Imaging assessment of these mucinous lesions is difficult, and recent studies have explored the use of tumor markers as clinical tools in evaluation of mucinous carcinoma of the appendix.

Carmignani et al (2004) evaluated patients with synchronous systemic and intraperitoneal dissemination of appendix cancer treated with cytoreductive surgery and perioperative regional chemotherapy with a mean follow up time of 42.6 months. Results of this study indicated that patients with elevated CEA and CA 19-9 levels had a shorter median survival time (p=0.0083 and p = 0.0193, respectively). In a subsequent study, Carmignani et al (2004) prospectively recorded tumor markers CEA and CA19-9 within 1 week prior to definitive treatment. The investigators found CEA elevated in 56% of 532 patients and CA19-9 elevated in 67.1% of those patients. They reported that “although the absolute level of tumor marker did not correlate with prognosis, a normal value indicated an improved survival.” Their findings included an elevated CEA in 35.2% of 110 patients determined to have recurrent disease and an elevated CA 19-9 in 62.9%, while 68.2% of patients had at least one of the tumor markers elevated.

Current guidelines indicate that for liver transplantation for primary sclerosing cholangitis, stringent efforts should be made to detect superadded cholangiocarcinoma, including measurement of CA 19-9 (Devlin & O'Grady, 1999).

Carmignani et al (2004a) conducted a study to report the role of combined treatments, including cytoreductive surgery and perioperative regional chemotherapy, in patients with synchronous systemic and intraperitoneal dissemination of appendix cancer. Study subjects were treated with cytoreductive
surgery and perioperative regional chemotherapy and statistical analysis of variables utilized survival as an end point and included demographic characteristics, prior surgical score (PSS), tumor marker levels, peritoneal cancer index (PCI), and completeness of cytoreduction (CC). With a mean follow-up of 42.6 months, median survival time (MST) for 15 patients was 28 months and 5-year survival rate was 29.4%. Female patients had a longer MST than male patients (p = 0.0199) and survival was better in patients with PSS 0 and 1 (p = 0.0277). Patients with elevated CEA and CA 19-9 levels had a shorter MST (p = 0.0083 and p = 0.0193, respectively) while PCI and CC comparisons did not show significant differences. The morbidity rate (n = 2) was 13.3% and the mortality (n = 2) rate was also 13.3%. The authors concluded that “acceptable morbidity and mortality and a 29.4% 5-year survival rate allows cytoreductive surgery and regional chemotherapy to be considered as a treatment option for selected patients with synchronous systemic and intraperitoneal dissemination of appendix cancer.”

Carmignani et al (2004b) in a further publication regarding gastrointestinal cancer, stated that carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9) tumor markers have found selected clinical application. The authors remarked that the use of these tumor markers in mucinous epithelial tumors of the appendix has not been previously determined. Thus, the authors conducted a study in which, in patients with peritoneal dissemination of a mucinous epithelial malignancy of the appendix, tumor markers CEA and CA 19-9 were prospectively recorded preoperatively within 1 week prior to definitive treatment and if the appendiceal tumor recurred, the tumor marker was determined. The primary endpoint was the accuracy of these two tumor markers in the management of this disease for these two specific clinical situations. CEA was elevated in 56% of 532 patients and CA 19-9 was elevated in 67.1% of these patients. Although the absolute level of tumor marker did not correlate with prognosis, a normal value indicated an improved survival. CEA was elevated in 35.2% of 110 patients determined to have recurrent disease and CA 19-9 was elevated in 62.9%. At least one of the tumor markers was elevated in 68.2% of patients. An elevated CEA tumor marker at the time of recurrence
indicated a reduced prognosis and both CEA and CA 19-9 tumor markers were elevated in a majority of these patients. This should be a valuable diagnostic tool previously underutilized in this group of patients. These tumor markers were also of benefit in the assessment of prognosis in that a normal level indicated an improved prognosis. At the time of a reoperative procedure, CEA and CA 19-9 tumor markers gave information regarding the progression of disease and have practical value in the management of epithelial appendiceal malignancy with peritoneal dissemination.

Andreopoulou et al (2007) stated that mucinous carcinoma of the appendix is a rare entity with a distinct natural history that poses diagnostic and therapeutic challenges and that mucinous peritoneal carcinomatosis is most commonly associated with primary tumors of the appendix and colon. The authors stated that usually the spread remains confined to the abdominal cavity and that imaging assessment of these mucinous lesions is difficult, while tumor markers (CEA and CA19.9) may be surrogates for extent of disease.

Recruitment for large scale studies given the rare nature of mucinous appendiceal carcinoma would be challenging. However, available evidence does illustrate a benefit to use of CA 19-9 in patients with mucinous appendiceal carcinoma.

Cathepsins

This enzyme plays a critical role in protein catabolism and tissue remodeling (Chin, et al., 2006). Over-expression is associated with non-ductal carcinoma and metastasis at the time of breast cancer diagnosis. High levels may have clinical significance in predicting decreased metastasis-free survival and decreased overall survival in women with node-negative breast cancer.

Svatek et al (2008) examined the role of urinary cathepsin B and L in the detection of bladder urothelial cell carcinoma. These investigators concluded that urinary cathepsin L is an independent predictor of bladder cancer presence and invasiveness in patients with a history of urothelial carcinoma of
the bladder. They stated that further evaluation of this marker is necessary before its use as an adjunct to cystoscopy for urothelial carcinoma of the bladder.

*CD 20*

CD 20 is used to determine eligibility for rituximab (Rituxan; anti-CD20) treatment in patients with B-cell non-Hodgkin's lymphomas (NHL) (Chin, et al., 2006). Rituximab is a genetically engineered, chimeric murine/human monoclonal antibody directed against the CD20 antigen found on the surface of normal and malignant B-cell lymphocytes. Since non-Hodgkin's Lymphoma (NHL) subtypes may differ in their response to rituximab, determination of drug sensitivity is important for choosing therapy.

*CD 25*

CD 25 is used to determine eligibility for denileukin diftitox treatment in patients with persistent or recurrent CTCL (Chin, et al., 2006). Denileukin diftitox (Ontak) is a cutaneous T-cell lymphoma (CTCL) therapy that targets the high-affinity interleukin-2 (IL-2) receptor. The IL-2 receptor may exist in a low-affinity form (CD25), an intermediate-affinity form (CD122/CD132), and a high-affinity form (CD25/CD122/CD132). Patients whose malignant cells express the CD25 component of the IL-2 receptor may respond to Ontak therapy.

*CD 33*

CD 33 is used to determine eligibility for gemtuzumab (Mylotarg, anti-CD33) treatment in patients with acute myeloid leukemia (Chen, et al., 2006). Gemtuzumab consists of a recombinant, humanized IgG kappa antibody conjugated to a cytotoxic anti-tumor antibiotic, calicheamicin, which binds specifically to the CD33 antigen. This antigen is found on the surface of leukemic blasts and immature normal cells of myelomonocytic lineage, but not in normal hematopoietic stem cells.

*CD 52*
CD 52 is used to determine eligibility for alemtuzumab (Campath, anti-CD52) treatment in patients with chronic lymphocytic leukemia (Chen, et al., 2006). CD52 is an antigen that can be expressed at high density on the surface of malignant CLL cells. Alemtuzumab is a humanized antibody targeted against CD52 and its binding is necessary for cell death and therapeutic response.

**CD 117, c-kit**

CD 117 is used to determine eligibility for treatment with imatinib mesylate in patients with c-kit-positive gastrointestinal stromal tumors (GISTs) (Chen, et al., 2006). The glycoprotein c-kit (CD117) is a member of the receptor tyrosine kinase subclass III family and has been implicated in a number of malignancies. Imatinib mesylate, a tyrosine kinase inhibitor, is effective in treating GISTs and other tumors that express c-kit.

**HCG**

Human chorionic gonadotropin (HCG) is normally produced in increasing quantities by the placenta during pregnancy. Accepted guidelines provide that HCG levels can be used to screen for choriocarcinoma in women who are at high risk for the disease, and to monitor the treatment of trophoblastic disease. The literature states that elevated HCG levels may also indicate the presence of cancers of the testis, ovary, liver, stomach, pancreas, and lung.

Accepted guidelines provide that alpha fetoprotein (AFP) and b-HCG measurements are valuable for determining prognosis and monitoring therapy in patients with non-seminomatous germ cell cancer. Because of the low incidence of elevated AFP and b-HCG levels in early-stage cancer, the literature suggests these markers have no value in screening for testicular cancer. However, the specificity of these markers is such that when determined simultaneously, at least one marker will be positive in 85% of patients with active cancer. The value of AFP and b-HCG as markers is enhanced by a low frequency of false-positive results and by the chemoresponsiveness of testicular cancer. The literature states that only rarely do patients with other types of
cancer have elevated levels of AFP. Non-cancerous conditions that can cause elevated AFP levels include benign liver conditions, such as cirrhosis or hepatitis, ataxia telangiectasia, Wiscott Aldrich syndrome, and pregnancy.

**AFP**

Alpha-fetoprotein (AFP) is a protein that is normally elevated in pregnant women since it is produced by the fetus; however, AFP is not usually found in the blood of adults. In men and in women who are not pregnant, an elevated level of AFP may indicate liver, ovarian or testicular cancer.

Alpha-fetoprotein is normally produced by a developing fetus. Alpha fetoprotein levels begin to decrease soon after birth and are usually undetectable in the blood of healthy adults, except during pregnancy. According to accepted guidelines, an elevated level of AFP strongly suggests the presence of either primary liver cancer or germ cell cancer of the ovary or testicle. As AFP is an established test for the diagnosis and monitoring of hepatoma, it is used as a screening tool to rule out the presence of a liver neoplasm before considering liver transplantation. This is especially pertinent in cases (e.g., cirrhosis) where there is an increased risk of developing a primary liver tumor.

Elevated serum AFP levels are most closely associated with nonseminomatous testicular cancer and hepatocellular cancer (Chin, 2006). The rate of clearance from serum after treatment is an indicator of the effectiveness of therapy. Conversely, the growth rate of progressive disease can be monitored by serially measuring serum AFP concentrations over time.

**ER, PR**

Estrogen receptor (ER) and progesterone receptor (PR) predicts response to hormone therapy for women with advanced breast cancer and those receiving adjuvant treatment, and prognosticates the aggressiveness of a tumor (Chin, 2006).

The estrogen receptor and progesterone receptor are intracellular
receptors that are measured directly in tumor tissue. These receptors are polypeptides that bind their respective hormones, translocate to the nucleus, and induce specific gene expression. Breast cancers are dependent upon estrogen and/or progesterone for growth and this effect is mediated through ERs and progesterone receptors (ER/PR) (Chin, et al., 2006). Both receptors may be over-expressed in malignant breast tissue. Most oncologists have used the estrogen receptor and also the progesterone receptor not only to predict the probability of response to hormonal therapy at the time of metastatic disease, but also to predict the likelihood of recurrent disease, and to predict the need for adjuvant hormonal therapy or chemotherapy. Although these latter uses for estrogen and progesterone receptors are commonly accepted by most oncologists, the data on which these conclusions are based are controversial.

**NSE**

Neuron-specific enolase (NSE) has been detected in patients with neuroblastoma, small cell lung cancer, Wilms' tumor, melanoma, and cancers of the thyroid, kidney, testicle, and pancreas. However, studies of NSE as a tumor marker have concentrated primarily on patients with neuroblastoma and small cell lung cancer. According to the available literature, measurement of NSE level in patients with these diseases cannot be correlated to the extent of the disease, the patient's prognosis, or the patient's response to treatment because of the poor sensitivity of this marker.

**LASA**

LASA is a complex marker that measures the amount of sialic acid in serum and can be elevated in serum from patients with any number of different neoplasms. Elevations in blood LASA levels have been reported in patients with mammary (63 percent), gastroenteric (65 percent), pulmonary (79 percent), and ovarian (94 percent) neoplasms as well as those with leukemia (86 percent), lymphoma (87 percent), melanoma (84 percent), sarcoma (97 percent), and Hodgkin disease (91 percent). As a
result, this assay may not have high specificity or sensitivity necessary for cancer detection (Chen, et al., 2006). This serum cancer marker has not been widely accepted for use in the detection or prognosis of colorectal carcinoma. There is no practical information concerning outcome and the use of LASA in the medical literature. Although several articles describe the use of LASA in the diagnosis of colorectal cancer and its association with tumor-node-metastasis (TNM) stage, it has been shown that patients with colorectal polyps and colorectal carcinoma both have elevated LASA levels, and that the levels returned to baseline after removal of either polyps or carcinomas.

**p53**

p53 is a tumor suppressor gene on the short arm of chromosome 17 that encodes a protein that is important in the regulation of cell division. Although the full role of p53 in the normal and neoplastic cell is unknown, there is evidence that the gene product is important in preventing the division of cells containing damaged DNA. p53 gene deletion or mutation is a frequent event along with other molecular abnormalities in colorectal carcinogenesis. The literature on p53 abnormality and prognosis in colorectal cancer suffers from a paucity of reported data and the use of a variety of techniques in assay and statistical analysis in the small numbers of cases analyzed. For these reasons, the literature generally does not recommend p53 analysis as a routine approach to assisting in the management of patients with colorectal cancer.

Guidelines from the American Society for Clinical Oncology (2016) recommend against the use of p53 to guide adjuvant chemotherapy in breast cancer. This is a moderate-strength recommendation based upon intermediate-quality evidence.

**Zap-70**

Zeta-chain-associated protein kinase 70, which is used as a prognostic marker in (CLL).

Zap-70 is indicated to assess prognosis and need for aggressive
therapy in patients with chronic lymphocytic leukemia (CLL) (Chin, et al., 2006). ZAP-70 is a 70-kD member of the Syk family of protein tyrosine kinases. It is expressed primarily in T-cells and natural killer (NK) cells and is critical for signal transduction following T-cell receptor engagement. In CLL B-cells, elevated ZAP-70 expression appears to predict the need for therapy as effectively as IgVH mutation status. Although ZAP-70 expression is strongly correlated with IgVH mutation status, the combination of the two markers may provide greater prognostic value than either marker alone. Positive ZAP-70 results predict an aggressive disease course.

*uPA*

The serine protease urokinase-type plasminogen activator (uPA) and its primary inhibitor, plasminogen activator inhibitor-1 (PAI-1), have shown promise for risk assessment and prediction of therapeutic response in primary breast cancer (Chin, et al., 2006). High levels of uPA or PAI-1 in primary tumor tissue are associated with an aggressive disease course and poor prognosis in both node-positive and node-negative breast cancer.

A report by the Belgian Healthcare Knowledge Centre (KCE) (San Miguel, et al., 2015) found no studies reporting on the impact of uPA/PAI-1 on clinical management (clinical utility).

Guidelines from the American Society for Clinical Oncology (2016) state: "If a patient has ER/PgR-positive, HER2-negative (node-negative) breast cancer, the clinician may use urokinase plasminogen activator and plasminogen activator inhibitor type 1 to guide decisions on adjuvant systemic therapy." This is a weak recommendation based upon high-quality evidence. The ASCO guidelines recommend the use of urokinase plasminogen activator and plasminogen activator inhibitor type 1 to guide decisions on adjuvant systemic therapy in patients with HER2-positive breast cancer or TN breast cancer.

*IgVh Mutation Status*

Chronic lymphocytic leukemia (CLL) patients can be divided into
two basic groups on the basis of the mutational status of the immunoglobulin heavy-chain variable-region (IgVH) gene in leukemic cells (Chin, 2006). Patients with IgVH mutations have longer survival than those without IgVH mutation. Thus, mutation analysis may be useful for planning management strategies.

*Kappa/Lambda Light Chain*

Elevated serum levels of monoclonal free light chains are associated with malignant plasma cell proliferation (e.g., multiple myeloma), primary amyloidosis, and light chain deposition disease (Chen, et al., 2006). The appearance of higher levels of free light chains in the urine may be indicative of kidney disease or malignant lymphoproliferative disease such as multiple myeloma. These tests have been used for the detection of multiple myeloma.

**KRAS**

The ras proto-oncogenes are normal cellular components, which are thought to be important for transduction of signals required for proliferation and differentiation. The ras oncogene family has three members: H-ras, K-ras, and N-ras. Ras gene mutations can be found in a variety of tumor types, although the incidence varies greatly. The highest incidences are found in adenocarcinomas of the pancreas (90 %), colon (50 %), and lung (30 %); thyroid tumors (50 %), and myeloid leukemia (30 %).

Investigators have established an association between some genotypes of K-ras (KRAS) oncogenes and response to treatment with cetuximab or panitumumab (Lievre et al, 2006 and 2008; Di Fiore et al, 2007; Gonçalves et al, 2008; De Roock et al, 2008). Patients whose tumors express specific forms of the KRAS gene exhibit considerably decreased responses to cetuximab and panitumumab. It has been theorized that cetuximab and panitumumab do not target epidermal growth factor receptor (EGFR) associated with these specific KRAS mutations and thus are unable to block their activation. It has been suggested that KRAS genotype be considered as a selection factor for cancer patients who are candidates for treatment with cetuximab or
Karapetis and colleagues (2008) stated that treatment with cetuximab improves overall survival (OS) and progression-free survival (PFS) and preserves the quality of life in patients with colorectal cancer that has not responded to chemotherapy. The mutation status of the K-ras gene in the tumor may affect the response to cetuximab and have treatment-independent prognostic value. These investigators analyzed tumor samples, obtained from 394 of 572 patients (68.9 %) with colorectal cancer who were randomly assigned to receive cetuximab plus best supportive care or best supportive care alone, to look for activating mutations in exon 2 of the K-ras gene. They evaluated if the mutation status of the K-ras gene was associated with survival in the cetuximab and supportive-care groups. Of the tumors evaluated for K-ras mutations, 42.3 % had at least one mutation in exon 2 of the gene. The effectiveness of cetuximab was significantly associated with K-ras mutation status ($p = 0.01$ and $p < 0.001$ for the interaction of K-ras mutation status with OS and PFS, respectively). In patients with wild-type K-ras tumors, treatment with cetuximab as compared with supportive care alone significantly improved OS (median of 9.5 versus 4.8 months; hazard ratio for death, 0.55; 95 % confidence interval [CI], 0.41 to 0.74; $p < 0.001$) and PFS (median of 3.7 months versus 1.9 months; hazard ratio for progression or death, 0.40; 95 % CI, 0.30 to 0.54; $p < 0.001$). Among patients with mutated K-ras tumors, there was no significant difference between those who were treated with cetuximab and those who received supportive care alone with respect to OS (hazard ratio, 0.98; $p = 0.89$) or PFS (hazard ratio, 0.99; $p = 0.96$). In the group of patients receiving best supportive care alone, the mutation status of the K-ras gene was not significantly associated with OS (hazard ratio for death, 1.01; $p = 0.97$). The authors concluded that patients with a colorectal tumor bearing mutated K-ras did not benefit from cetuximab, whereas patients with a tumor bearing wild-type K-ras did benefit from cetuximab. The mutation status of the K-ras gene had no influence on survival among patients treated with best supportive care alone.

The ASCO’s provisional clinical opinion on testing for KRAS gene
mutations in patients with metastatic colorectal carcinoma to predict response to anti-EGFR monoclonal antibody therapy (Allegra et al, 2009) stated that based on systematic reviews of the relevant literature, all patients with metastatic colorectal carcinoma who are candidates for anti-EGFR antibody therapy should have their tumor tested for KRAS mutations in a CLIA-accredited laboratory. If KRAS mutation in codon 12 or 13 is detected, then patients with metastatic colorectal carcinoma should not receive anti-EGFR antibody therapy as part of their treatment.

The KRAS oncogene mutation tests are intended to aid in the formulation of treatment decisions for patients who may be candidates for treatment of metastatic epithelial cancers with anti-EGFR therapies such as cetuximab or panitumumab. Several tests for KRAS mutation are currently available in the United States; however, at this time, no KRAS genotype test kits have been approved by the FDA.

At the 2008 Annual Meeting of the American Society of Clinical Oncology (ASCO), data on 540 patients with metastatic colorectal cancer in the randomized, phase III CRYSTAL trial were presented. Among 192 patients with KRAS mutations, there was no improvement in overall responses or PFS from the addition of cetuximab to standard chemotherapy. In the patients with normal KRAS, the 1-year PFS rate was 43 % for patients receiving cetuximab versus 25 % for those receiving only standard chemotherapy, and the overall response rate was 59 % versus 43 %, respectively (van Cutsem, 2008). Also at the 2008 ASCO meeting, data from 233 metastatic colorectal cancer patients were presented that confirmed the correlation of KRAS status with patient response to anti-EGFR therapy. No benefit was found after addition of cetuximab to standard chemotherapy with FOLFOX (the combination of fluorouracil, leucovorin, and oxaliplatin) in patients with a mutated KRAS; however, addition of cetuximab to FOLFOX increased both response rate and PFS in patients with a wild-type (i.e., un-mutated) KRAS gene (Bokemeyer, 2008). Response to panitumumab was correlated to KRAS status in a published phase III trial. A total of 427 patients with metastatic colorectal cancer received either panitumumab
Panitumumab exhibited a 17% response rate among patients with normal KRAS, but 0% response among patients with KRAS mutations (Amado, 2008).

A meta-analysis of results from 8 studies involving 817 patients with colorectal cancer found that the presence of KRAS mutation predicted lack of response to treatment with anti-EGFR monoclonal antibodies (e.g., panitumumab or cetuximab), whether as stand-alone therapy or in combination with chemotherapy (Linardou et al, 2008). This analysis also provided empirical evidence that k-RAS mutations are highly specific negative predictors of response (de-novo resistance) to single-agent EGFR tyrosine-kinase inhibitors in advanced non-small cell lung cancer; and similarly to anti-EGFR monoclonal antibodies alone or in combination with chemotherapy in patients with metastatic colorectal cancer.

The Blue Cross and Blue Shield Association (BCBSA, 2008) Technology Evaluation Center Medical Advisory Panel concluded that use of KRAS mutation analysis meets TEC criteria to predict non-response to anti-EGFR monoclonal antibodies cetuximab and panitumumab to treat metastatic colorectal cancer. The TEC assessment found that the evidence is sufficient to conclude that patients with mutated KRAS tumors in the setting of metastatic colorectal cancer do not respond to anti-EGFR monoclonal antibody therapy. The assessment explained that the data show that the clinical benefit of using EGFR inhibitors in treating metastatic colorectal cancer, either as monotherapy or in combination with other treatment regimens, is not seen in patients with KRAS-mutated tumors. The assessment found: "This data supports knowing a patient’s tumor mutation status before consideration of use of an EGFR inhibitor in the treatment regimen. Identifying patients whose tumors express mutated KRAS will avoid exposing patients to ineffective drugs, avoid exposure to unnecessary drug toxicities, and expedite the use of the best available alternative therapy."

Colorectal cancer guidelines from the National Comprehensive Cancer Network (NCCN, 2010) recommend consideration of reflex BRAF testing in patients with wild type KRAS. The NCCN
guidelines explain that several small studies suggest that patients with wild-type KRAS and a BRAF mutation are unlikely to respond to anti-EGFR therapies such as cetuximab and panitumumab. The guidelines explain that patients with a known BRAF mutation are unlikely to respond to anti-EGFR antibodies, although the data are somewhat inconsistent. Studies demonstrate that in patients with metastatic colorectal cancer, about 8 percent have mutations in the BRAF gene. Testing for the BRAF V600E mutation is performed by PCR amplification and direct DNA sequence analysis.

Ratner et al (2010) stated that ovarian cancer (OC) is the single most deadly form of women's cancer, typically presenting as an advanced disease at diagnosis in part due to a lack of known risk factors or genetic markers of risk. The KRAS oncogene and altered levels of the microRNA (miRNA) let-7 are associated with an increased risk of developing solid tumors. In this study, these researchers investigated a hypothesized association between an increased risk of OC and a variant allele of KRAS at rs61764370, referred to as the KRAS-variant, which disrupts a let-7 miRNA binding site in this oncogene. Specimens obtained were tested for the presence of the KRAS-variant from non-selected OC patients in 3 independent cohorts, 2 independent ovarian case-control studies, and OC patients with hereditary breast and ovarian cancer syndrome (HBOC) as well as their family members. The results indicated that the KRAS-variant is associated with more than 25% of non-selected OC cases. Furthermore, these researchers found that it is a marker for a significant increased risk of developing OC, as confirmed by 2 independent case-control analyses. Lastly, they determined that the KRAS-variant was present in 61% of HBOC patients without BRCA1 or BRCA2 mutations, previously considered uninformative, as well as in their family members with cancer. These findings supported the hypothesis that the KRAS-variant is a genetic marker for increased risk of developing OC, and they suggested that the KRAS-variant may be a new genetic marker of cancer risk for HBOC families without other known genetic abnormalities.

Hollestelle et al (2011) noted that recently, a variant allele in the 3'UTR of the KRAS gene (rs61764370 T>G) was shown to be associated with an increased risk for developing non-small cell
lung cancer, as well as OC, and was most enriched in OC patients from HBOC families. This functional variant has been shown to disrupt a let-7 miRNA binding site leading to increased expression of KRAS in vitro. In the current study, these investigators genotyped this KRAS-variant in breast cancer index cases from 268 BRCA1 families, 89 BRCA2 families, 685 non-BRCA1/BRCA2 families, and 797 geographically matched controls. The allele frequency of the KRAS-variant was found to be increased among patients with breast cancer from BRCA1, but not BRCA2 or non-BRCA1/BRCA2 families as compared to controls. As BRCA1 carriers mostly develop ER-negative breast cancers, these researchers also examined the variant allele frequency among indexes from non-BRCA1/BRCA2 families with ER-negative breast cancer. The prevalence of the KRAS-variant was, however, not significantly increased as compared to controls, suggesting that the variant allele not just simply associates with ER-negative breast cancer. Subsequent expansion of the number of BRCA1 carriers with breast cancer by including other family members in addition to the index cases resulted in loss of significance for the association between the variant allele and mutant BRCA1 breast cancer. In this same cohort, the KRAS-variant did not appear to modify breast cancer risk for BRCA1 carriers. More importantly, results from the current study suggested that KRAS-variant frequencies might be increased among BRCA1 carriers, but solid proof requires confirmation in a larger cohort of BRCA1 carriers.

Therascreen KRAS RGQ PCR Kit (Qiagen) is intended to detect 7 mutations in codons 12 and 13 of the KRAS gene (Raman, et al., 2013). The kit utilizes two technologies — ARMS and Scorpions — for detection of mutations in real-time PCR. The therascreen KRAS RGQ PCR kit is being developed as a companion diagnostic to aid clinicians, through detection of KRAS mutations, in the identification of patients with metastatic colorectal cancer (mCRC) who are more likely to benefit from cetuximab.

PreOvar™ tests (Mira Dx) for the KRAS-variant, and will help identify ovarian cancer patients whose female relatives should also be evaluated for the KRAS-variant (Raman, et al., 2013). PreOvar™ may also help assess the relative risk of developing ovarian cancer for women who have a family history of ovarian
cancer without a living proband (ancestor with the disease). The KRAS-variant is present in 6-10% of the general population and 25% of non-selected women with epithelial ovarian cancer. Additionally, the KRAS-variant was identified in over 60% of Hereditary Breast and Ovarian Cancer (HBOC) patients that were previously classified as “uninformative,” or negative for other known genetic markers of ovarian cancer risk. The test determines if KRAS-variant may put someone at increased risk for developing ovarian cancer.

The Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group (EWG) (2013) found that, for patients with metastatic colorectal cancer (mCRC) who are being considered for treatment with cetuximab or panitumumab, there is convincing evidence to recommend clinical use of KRAS mutation analysis to determine which patients are KRAS mutation positive and therefore unlikely to benefit from these agents before initiation of therapy. The level of certainty of the evidence was deemed high, and the magnitude of net health benefit from avoiding potentially ineffective and harmful treatment, along with promoting more immediate access to what could be the next most effective treatment, is at least moderate.

The EWG found insufficient evidence to recommend for or against BRAF V600E testing for the same clinical scenario (EGAPP, 2013). The level of certainty for BRAF V600E testing to guide antiepidermal growth factor receptor (EGFR) therapy was deemed low. The EWG encourages further studies of the potential value of testing in patients with mCRC who were found to have tumors that are wild type (mutation negative) for KRAS to predict responsiveness to therapy.

**Bladder Cancer: BTA-stat, NMP22, Urovysion, ImmunoCyt**

In the United States, bladder malignancy is the 4th commonest cancer in men and the 8th commonest in women. Patients usually present with urinary tract symptoms (e.g., gross or microscopic hematuria or irritative voiding symptoms such as frequency, dysuria, and urgency). Evaluations of these patients usually entail voided-urine cytology, cystoscopy, and upper
urinary tract imaging such as intravenous pyelography, renal sonography, or retrograde pyelography. Most newly diagnosed bladder cancers are superficial (i.e., not invading beyond the lamina propria on histological examination), and are known as transitional cell carcinoma (TCC). These superficial bladder cancers are usually managed by transurethral resection. However, the literature shows that approximately 50 to 75 % of treated TCC recur. Furthermore, 10 to 15 % of TCC progress to muscle-invasive bladder cancer. According to the literature, the prevalence of recurrence after initial treatment as well as the natural history of TCC necessitates long-term follow-up. Following treatment, accepted guidelines provide that patients who have previously been diagnosed with TCC should usually undergo urine cytology/cystoscopy every 3 months in the 1st year, every 6 months in the 2nd year, and once-yearly afterwards.

Currently, urine cytology with confirmatory cystoscopy represents the cornerstone for the identification of bladder tumors. However, the subjectivity and low sensitivity of cytology led to the development of several urine-based tests as adjuncts to cytology/cystoscopy for the diagnosis and follow-up of patients with TCC. These tests include the BTA Stat test (Bard Diagnostic, Redmond, WA), the NMP22 test (Matritech, Newton, MA), the Aura-Tek FDP test (PerImmune, Rockville, MD), and the Vysis UroVysion FISH Test (Vysis, Inc., Downers Grove, IL). They are usually objective, qualitative (BTA Stat and Aura-Tek FDP), or quantitative (NMP22, UroVysion), and have higher sensitivity than cytology, but some have lower specificity. So far, no single bladder tumor marker has emerged as the generally accepted test of choice, and none has been established as a screening tool for bladder malignancy.

Urine-based markers, such as proteins with increased cancer cell expression or chromosomal abnormalities in the urine, may be detected using a variety of laboratory methods to aid in the management of bladder cancer. The following markers/tests are currently available:

- Bladder tumor antigen (BTA) (eg, BTA stat and BTA TRAK)
- Fluorescence immunocytology (eg, ImmunoCyt/uCyt+)
Fluorescence in situ hybridization (FISH) (eg, UroVysion)
- mRNA quantification by RT-qPRC testing (eg, Cxbladder)
- Nuclear matrix protein 22 (NMP22) (eg, NMP22 BladderChek and Matritech NMP22 Test)

Urine-based markers have a role in the detection of bladder cancer recurrence in individuals with a history of bladder cancer and are used adjunctively with urinary cytology and cystoscopy. These tests have also been proposed for bladder cancer screening, diagnosis of bladder cancer in individuals symptomatic of bladder cancer and for the evaluation of hematuria.

The UroVysion Bladder Cancer Kit (UroVysion Kit) (Baycare Laboratories) is designed to detect aneuploidy for chromosomes 3, 7, 17, and loss of the 9p21 locus via fluorescence in situ hybridization (FISH) in urine specimens from persons with hematuria suspected of having bladder cancer (Raman, et al., 2013). FISH analysis is used in conjunction with cystoscopy to monitor for recurrence among those with previously diagnosed bladder cancer. FISH analysis is a surveillance tool in established primary and secondary bladder adenocarcinoma.

The ImmunoCyt is an immunocytochemistry assay for the detection of tumor cells shed in the urine of patients previously diagnosed with bladder cancer (Chen, et al., 2006). This test is intended to augment the sensitivity of cytology for the detection of tumor cells in the urine of individuals previously diagnosed with bladder cancer. The test has been used for detection of tumor cells in the urine of individuals previously diagnosed with bladder cancer, and for use in conjunction with cystoscopy as an aid in the management of bladder cancer.

Although urine cytology has been shown to be less accurate than urinary biomarker tests, familiarity with the method as well as ease of performance justify the continued routine use of the former by primary care physicians, especially in patients who have no history of bladder malignancy. The urine-based biomarker tests have been shown to be accurate in detecting low-grade bladder tumors. In particular, these tests may be of help in deciding the need for further diagnostic assessment of patients
with a history of bladder cancer and negative results on urine cytology. For example, elevated levels of urinary bladder tumor markers in patients with a history of TCC may warrant earlier, rather than delayed, cystoscopic examination. On the other hand, consideration may be given to lengthening the intervals between cystoscopic investigations when values of these tumor markers are normal.

An assessment by the Adelaide Health Technology Assessment (Mundy & Hiller, 2009) concluded that the NMP BladderCheck and UroVysion FISH assay, designed for the detection of bladder cancer in high risk patients, have poor sensitivity and poor positive predictive values. The assessment recommended that these assays not be used in asymptomatic patients. The assessment suggested, however, that these tests may be useful in the monitoring of patients with transitional cell carcinoma between cystoscopies. The AHTA recommended that this technology not be assessed further.

An assessment prepared for the Agency for Healthcare Research and Quality (Meleth, et al., 2014) found: "Although UroVysion is marketed as a diagnostic rather than a prognostic test, limited evidence from two small studies (total N=168) rated as low or medium risk of bias supported associations between test result and prognosis for risk of recurrence. We found no studies that directly assessed the impact of a test of interest on both physician decision-making and downstream health outcomes to establish clinical utility. We attempted to construct an indirect chain of evidence to answer the overarching question, but we were unable to do so. Even in the cases where the tests seemed to add value in determining prognosis (i.e., evidence of clinical validity), we found no evidence that using the test was related to improved outcomes for patients."

The American Urologic Association's guideline on “Diagnosis, evaluation and follow-up of asymptomatic microhematuria (AMH) in adults” (Davis et al, 2012) stated that “The use of urine cytology and urine markers (Nuclear Matrix Protein 22 [NMP22], bladder tumor antigen [BTA]-stat, and UroVysion fluorescence in situ hybridization assay [FISH]) is not recommended as a part of
the routine evaluation of the asymptomatic microhematuria patient."

Chou et al (2015) systematically reviewed the evidence on the accuracy of urinary biomarkers for diagnosis of bladder cancer in adults who have signs or symptoms of the disease or are undergoing surveillance for recurrent disease. Data sources included Ovid MEDLINE (January 1990 through June 2015), Cochrane Central Register of Controlled Trials, Cochrane Database of Systematic Reviews, and reference lists. A total of 57 studies that evaluated the diagnostic accuracy of quantitative or qualitative nuclear matrix protein 22 (NMP22), qualitative or quantitative bladder tumor antigen (BTA), FISH, fluorescent immunohistochemistry (ImmunoCyt [Scimedx]), and Cxbladder (Pacific Edge Diagnostics USA) using cystoscopy and histopathology as the reference standard met inclusion criteria; case-control studies were excluded. Dual extraction and quality assessment of individual studies were carried out; overall strength of evidence (SOE) was also assessed. Across biomarkers, sensitivities ranged from 0.57 to 0.82 and specificities ranged from 0.74 to 0.88. Positive likelihood ratios ranged from 2.52 to 5.53, and negative likelihood ratios ranged from 0.21 to 0.48 (moderate SOE for quantitative NMP22, qualitative BTA, FISH, and ImmunoCyt; low SOE for others). For some biomarkers, sensitivity was higher for initial diagnosis of bladder cancer than for diagnosis of recurrence. Sensitivity increased with higher tumor stage or grade. Studies that directly compared the accuracy of quantitative NMP22 and qualitative BTA found no differences in diagnostic accuracy (moderate SOE); head-to-head studies of other biomarkers were limited. Urinary biomarkers plus cytologic evaluation were more sensitive than biomarkers alone but missed about 10% of bladder cancer cases. The authors concluded that urinary biomarkers miss a substantial proportion of patients with bladder cancer and are subject to false-positive results in others; accuracy is poor for low-stage and low-grade tumors. They stated that research is needed to understand how the use of these biomarkers with other diagnostic tests affect the use of cystoscopy and clinical outcomes.
In an editorial that accompanied the afore-mentioned study, Abbosh and Plimack (2015) stated that “Until urinary biomarkers become available that are sufficiently accurate to supplant the current recommended detection algorithms in biomarker-negative patients, they will not be a cost-effective addition to strategies to detect bladder cancer”.

In summary, urine-based bladder tumor marker tests have been shown to be useful as an adjunct to urine cytology and cystoscopy in monitoring for recurrences of bladder cancer, but according to the available literature should not be used as a screening tool for bladder malignancy. The U.S. Preventive Services Task Force (USPSTF, 2004) has concluded that the potential harms of screening for bladder cancer using available tests, such as microscopic urinalysis, urine dipstick, urine cytology, or such new tests as bladder tumor antigen (BTA) or nuclear matrix protein (NMP22) immunoassay, outweigh any potential benefits.

Cxbladder

O’Sullivan and colleagues (2012) examined if the RNA assay uRNA® and its derivative Cxbladder® have greater sensitivity for the detection of bladder cancer than cytology, NMP22™ BladderChek™ and NMP22™ ELISA, and whether they are useful in risk stratification. A total of 485 patients presenting with gross hematuria but without a history of urothelial cancer were recruited prospectively from 11 urology clinics in Australasia. Voided urine samples were obtained before cystoscopy. The sensitivity and specificity of the RNA tests were compared to cytology and the NMP22 assays using cystoscopy as the reference. The ability of Cxbladder to distinguish between low grade, stage Ta urothelial carcinoma and more advanced urothelial carcinoma was also determined. uRNA detected 41 of 66 urothelial carcinoma cases (62.1 % sensitivity, 95 % confidence interval [CI]: 49.3 to 73.8) compared with NMP22 ELISA (50.0 %, 95 % CI: 37.4 to 62.6), BladderChek (37.9 %, 95 % CI: 26.2 to 50.7) and cytology (56.1 %, 95 % CI: 43.8 to 68.3). Cxbladder, which was developed on the study data, detected 82 %, including 97 % of the high grade tumors and 100 % of tumors stage 1 or greater.
The cut-offs for uRNA and Cxbladder were pre-specified to give a specificity of 85%. The specificity of cytology was 94.5% (95% CI: 91.9 to 96.5), NMP22 ELISA 88.0%, (95% CI: 84.6 to 91.0) and BladderChek 96.4% (95% CI: 94.2 to 98.0). Cxbladder distinguished between low-grade Ta tumors and other detected urothelial carcinoma with a sensitivity of 91% and a specificity of 90%. The authors concluded that uRNA and Cxbladder showed improved sensitivity for the detection of urothelial carcinoma compared to the NMP22 assays. Stratification with Cxbladder provides a potential method to prioritize patients for the management of waiting lists.

An UpToDate review on “Clinical presentation, diagnosis, and staging of bladder cancer” (Lotan and Choueiri, 2013) does not mention the use of mRNA biomarkers/PCR testing as a management tool for bladder cancer. Furthermore, NCCN’s clinical practice guideline on “Bladder cancer” (Version 1.2014) does not mention the use of mRNA biomarkers/PCR testing as a management tool for bladder cancer.

An assessment of urinary biomarkers for diagnosis of bladder cancer prepared for the Agency for Healthcare Research and Quality (Chou, et al., 2016) identified only one study of Cxbladder meeting inclusion criteria, graded as moderate quality, with an overall strength of evidence of "low,"

**OncotypeDx Breast**

Oncotype Dx (Genomic Health, Inc., Redwood City, CA) is a diagnostic laboratory-developed assay that quantifies the likelihood of breast cancer recurrence in women with newly diagnosed, stage I or II, node negative, estrogen receptor positive breast cancer, who will be treated with tamoxifen. The assay analyzes the expression of a panel of 21 genes, and is intended for use in conjunction with other conventional methods of breast cancer analysis. Together with staging, grading, and other tumor marker analyses, Oncotype Dx is intended to provide greater insight into the likelihood of systemic disease recurrence. Clinical studies have evaluated the prognostic significance of the Oncotype Dx multigene assay in breast cancer (Paik et al, 2004;
Oncotype Dx analyses the patterns of 21 genes is being applied as a quantification tool for likelihood of breast cancer recurrence within 10 years of newly diagnosed, stage I or II, lymph node-negative, hormone receptor-positive breast cancer in women who will be treated with tamoxifen (Raman, et al., 2013). Oncotype is being applied as a quantification tool for likelihood of breast cancer recurrence in 10 years in women with newly diagnosed breast cancer. It is also intended to assist in making decisions regarding adjuvant chemotherapy based on recurrence likelihood.

There currently is a lack of evidence from prospective clinical studies of the impact of this test on the management of women with breast cancer demonstrating improvements in clinical outcomes (Lopez, et al., 2010; Romeo, et al., 2010; Tiwana, et al., 2013; IETS, 2013). Bast and Hortobagyi (2004) commented that “[b]efore use of the recurrence score [from the Oncotype Dx multigene assay] is applied to general patient care, however, additional studies are needed.” The National Cancer Institute is sponsoring a prospective, randomized controlled clinical study, the TAILORx study, using the Oncotype Dx assay to help identify a group of patients with a mid-range risk of recurrence to determine whether treating patients with hormonal therapy only is equivalent to treating them with hormonal therapy in combination with adjuvant chemotherapy.

However, there is indirect evidence of the clinical utility of the Oncotype Dx. Paik et al (2006) used banked tumor samples from previous clinical studies of tamoxifen and adjuvant chemotherapy in early breast cancer to assess the performance of the Oncotype Dx multigene assay in predicting response to adjuvant chemotherapy. These investigators examined tumor samples from subjects enrolled in the National Surgical Adjuvant Breast and Bowel Project (NSABP) B20 trial to determine whether there is a correlation between the recurrence score (RS) determined by Oncotype Dx in tumor samples and subsequent response to adjuvant chemotherapy. A total of 651 patients were assessable (227 randomly assigned to tamoxifen and 424 randomly assigned...
to tamoxifen plus chemotherapy). The test for interaction between chemotherapy treatment and RS was statistically significant ($p = 0.038$). Patients with high-RS (RS greater than or equal to 31) tumors (ie, high risk of recurrence) had a large benefit from chemotherapy (relative risk, 0.26; 95% confidence interval 0.13 to 0.53; absolute decrease in 10-year distant recurrence rate: mean, 27.6%; standard error, 8.0%). Patients with low-RS (less than 18) tumors derived minimal, if any, benefit from chemotherapy treatment (relative risk, 1.31; 95% confidence interval, 0.46 to 3.78; absolute decrease in distant recurrence rate at 10 years: mean, -1.1%; standard error, 2.2%). The investigators found that patients with intermediate-RS tumors did not appear to have a large benefit, but the investigators concluded that the uncertainty in the estimate cannot exclude a clinically important benefit.

One limitation of the study by Paik et al (2006) is that the NASBP B20 trial was conducted before the advent of important advances in breast cancer chemotherapy, including the introduction of trastuzumab (Herceptin), which has been demonstrated to improve overall and disease-free survival in breast cancer patients with HER2 positive tumors. Current guidelines recommend the use of trastuzumab adjuvant chemotherapy in women with metastatic HER2 positive breast cancer, and women with HER2 positive nonmetastatic breast cancers 1 cm or more in diameter. Thus, the Oncotype Dx score would not influence the decision to use adjuvant trastuzumab in women with HER2 positive tumors 1 cm or more in diameter.

Commenting on an early report of this study by Paik et al, of the Oncotype Dx presented in abstract form, the BlueCross BlueShield Association Technology Evaluation Center assessment stated that “additional studies in different populations are needed to confirm whether risk prediction is sufficiently accurate for physicians and patients to choose with confidence whether to withhold adjuvant chemotherapy.”

An international consensus group (Azim, et al., 2013) found the available evidence on the analytical and clinical validity of Oncotype Dx Breast to be convincing. However, neither the
Oncotype Dx or none of the other genomic tests the evaluated demonstrated robust evidence of clinical utility: they stated that it was not clear from the current evidence that modifying treatment decisions based on the results of a given genomic test could result in improving clinical outcome.

The selection criteria for the TailorRx prospective trial of OncotypeDx state that candidates should have negative axillary nodes as determined by a sentinel lymph node biopsy and/or axillary dissection as defined by the American Joint Committee on Cancer 6th Edition Staging System (NCI, 2009). The AJCC 6th Edition criteria redefined isolated tumor cells as node negative (the prior version of the criteria, AJCC 5th Edition, classified isolated tumor cells as node positive). "Isolated tumor cells (single cells or cell deposits) will now be defined as tumor cell deposits no larger than 0.2 mm in diameter that may or may not (but usually do not) show histologic evidence of malignant activity. Pending further information, isolated tumor cells will be classified as node-negative, because it is believed that the unknown benefits of providing treatment for these small lesions would not outweigh the morbidity caused by the treatment itself." (Singletary, et al., 2002). However, the banked tumor samples used in the study by Paik, et al. (2006) to validate the OncotypeDx were classified based on AJCC 5th Ed. criteria. In addition, there is new evidence demonstrating that women with isolated tumor cells are at a significantly increased risk of breast cancer. Investigators from the Netherlands found an association between isolated tumor cells and micrometastases in regional lymph nodes and clinical outcome of breast cancer (de Boer, et al., 2009). These investigators identified all patients in the Netherlands who underwent a sentinel-node biopsy for breast cancer before 2006 and had breast cancer with favorable primary-tumor characteristics and isolated tumor cells or micrometastases in the regional lymph nodes. Patients with node-negative disease were randomly selected from the years 2000 and 2001. The primary end point was disease-free survival. The investigators identified 856 patients with node-negative disease who had not received systemic adjuvant therapy (the node-negative, no-adjuvant-therapy cohort), 856 patients with isolated tumor cells or micrometastases who had not
received systemic adjuvant therapy (the node-positive, no-adjuvant-therapy cohort), and 995 patients with isolated tumor cells or micrometastases who had received such treatment (the node-positive, adjuvant-therapy cohort). The median follow-up was 5.1 years. The adjusted hazard ratio for disease events among patients with isolated tumor cells who did not receive systemic therapy, as compared with women with node-negative disease, was 1.50 (95% confidence interval [CI], 1.15 to 1.94); among patients with micrometastases, the adjusted hazard ratio was 1.56 (95% CI, 1.15 to 2.13). Among patients with isolated tumor cells or micrometastases, the adjusted hazard ratio was 0.57 (95% CI, 0.45 to 0.73) in the node-positive, adjuvant-therapy cohort, as compared with the node-positive, no-adjuvant-therapy cohort. The investigators concluded that isolated tumor cells or micrometastases in regional lymph nodes were associated with a reduced 5-year rate of disease-free survival among women with favorable early-stage breast cancer who did not receive adjuvant therapy. In patients with isolated tumor cells or micrometastases who received adjuvant therapy, disease-free survival was improved.

The Medical Advisory Panel of the BlueCross BlueShield Association Technology Evaluation Center (BCBCA, 2014) concluded that use of Oncotype DX to determine recurrence risk for deciding whether to undergo adjuvant chemotherapy in women with unilateral, nonfixed, hormone receptor–positive, lymph node–negative breast cancer who will receive hormonal therapy meets the Blue Cross and Blue Shield Association Technology Evaluation Center (TEC) criteria. A technology assessment by the BlueCross BlueShield Association (2014) stated: "Technical performance of the assay is well documented and is unlikely to be a major source of variability; rather, tissue sampling is likely the greatest source of variability. Retrospective epidemiologic analyses indicated strong, independent associations between Oncotype DX recurrence score (RS) result and distant disease recurrence or death from breast cancer. The evidence identified a subset of conventionally classified, high-risk patients who are at sufficiently low risk of recurrence by Oncotype DX that they might reasonably decide that the harms (toxicity) of chemotherapy outweigh the very
small absolute benefit. Two studies of the original validation data, in which conventionally classified patients were reclassified by Oncotype DX result, indicated that the test provides significant recurrence risk information in addition to conventional criteria for individual patient risk classification. Additional evidence indicated that Oncotype DX results are significantly associated with breast cancer death in a community-based patient population, and that RS high-risk patients benefit from chemotherapy, whereas benefits for other RS categories were not statistically significant. Thus, the evidence was judged sufficient to permit conclusions regarding probable health outcomes."

The Oncotype Dx has also been promoted for use in women with node-positive, ER-positive breast cancer. An assessment by the BlueCross BlueShield Association (2010) concluded that it has not yet been demonstrated whether use of the Oncotype Dx for selecting adjuvant chemotherapy in patients with lymph-node-positive breast cancer improves health outcomes. The report explained that the evidence for not selecting chemotherapy for women with low RS values is based on low event rates and wide confidence intervals that include the possibility of benefit from chemotherapy. Because the data allow for a possible benefit of chemotherapy in patients with low RS results, it is unknown if health outcomes would be improved, the same, or worse, if chemotherapy was withheld in these women. The report stated that, due to the lack of clear and sufficient information, there is a need for a second, confirmatory study. The report stated that the Fred Hutchinson Cancer Research Center will conduct a nationwide, NCI-sponsored, Phase III clinical trial to determine the predictive ability of the Oncotype Dx to identify which patients with lymph-node-positive breast cancer will benefit from chemotherapy.

The clinical evidence base for OncotypeDX is considered to be the most robust. There was some evidence on the impact of the test on decision-making and to support the case that OncotypeDX predicts chemotherapy benefit; however, few studies were UK based and limitations in relation to study design were identified. OncotypeDX has a more robust evidence base, but further evidence on its impact on decision-making in the UK and the
predictive ability of the test in an ER+, LN-, HER- population receiving current drug regimens is needed.

Guidelines from the National Comprehensive Cancer Network (NCCN, 2015) state that “the 21-gene RT-PCR assay recurrence score can be considered in select patients with 1-3 involved ipsilateral axillary lymph nodes to guide the addition of combination chemotherapy to standard hormone therapy. A retrospective analysis of a prospective randomized trial suggests that the test is predictive in this group similar to its performance in node-negative disease.” The NCCN guidelines (2015) explained: “Unplanned, retrospective subset analysis from a single randomized clinical trial in post-menopausal, ALN-positive, ER-positive breast cancer found that the 21-gene RT-PCR assay may provide predictive information for chemotherapy in addition to tamoxifen [citing Albain, et al., 2010]. Patients with a high score in the study benefited from chemotherapy, whereas patients with a low score did not appear to benefit from the addition of chemotherapy regardless of the number of positive lymph nodes. Patient selection for assay use remains controversial.” “The RxPONDER trial will confirm the SWOG-8814 trial data for women with ER-positive, node-positive disease treated with endocrine therapy with or without chemotherapy based on risk scores.”

Guidance from the National Institute for Health and Care Excellence (2013) stated: "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 assessed as being at intermediate risk; and information on the biological features of the cancer provided by Oncotype DX is likely to help in predicting the course of the disease and would therefore help when making the decision about prescribing chemotherapy; and the manufacturer provides Oncotype DX to National Health Service (NHS) organisations according to the confidential arrangement agreed with the National Institute for Health and Care Excellence (NICE). NICE encourages further data collection on the use of Oncotype DX in the NHS."
An assessment by the Belgian Healthcare Knowledge Center (KCE) (San Miguel, et al., 2015) concluded that "the evidence for Oncotype DX is more robust than the evidence for other tests."

The KCE Report noted, however, that important evidence gaps are still present. The KCE review mostly identified studies supporting the prognostic ability (clinical validity) of the test. The KCE judged these studies to be of moderate to high quality. The KCE found no prospective studies reporting on the impact of Oncotype DX on long-term outcomes such as overall survival, while four studies indicated that Oncotype DX leads to changes in decision making.

The KCE identified two studies on the predictive benefit of the test, one for lymph node patients. The KCE reported also noted that the first evidence relating to improvements in quality of life and reductions in patient anxiety as a result of using the test has been reported, but this is based on small patient numbers and further evidence is required.

Guidelines from the American Society for Clinical Oncology (2016) state: "If a patient has ER/PgR-positive, HER2-negative (node-negative) breast cancer, the clinician may use the 21-gene recurrence score (RS; Oncotype DX; Genomic Health, Redwood City, CA) to guide decisions on adjuvant systemic chemotherapy."

This is a strong recommendation based upon high quality evidence. The ASCO guidelines recommend against OncotypeDx Breast to guide decisions on adjuvant systemic chemotherapy for patients with ER/PgR-positive, HER2-negative (node-positive) breast cancer. The guidelines also recommend against the use of OncotypeDx Breast in women with HER2-positive breast cancer or TN breast cancer. The guidelines recommended against the use of OncotypeDx Breast to guide decisions on extended endocrine therapy for patients with ER/PgR-positive, HER-2 negative (node-negative) breast cancer who have had 5 years of endocrine therapy without evidence of recurrence.

Acceptance of 21-gene recurrence score assays as tools for clinical decision making in women or men with early stage breast cancer is controversial due to the lack of prospective validation studies, nevertheless, 2007 guidelines from an expert panel convened by ASCO on tumor markers in breast cancer concluded that multiparameter gene expression analysis (i.e., Oncotype Dx
assay) can be used to predict the risk of recurrence in women with newly diagnosed, node-negative, ER-positive breast cancer. Although it is reasonable to consider the use of a 21-gene recurrence score assay in males, none of the data generated to date have been in men with breast cancer (Gradishar, 2010).

A 2009 abstract that looked at cases of male breast cancer (BC) with Oncotype Dx, concluded, “This large genomic study of male BC reveals a heterogeneous biology as measured by the standardized quantitative oncotype Dx breast cancer assay, similar to that observed in female BC. Some differences, which may reflect the differences in hormone biology between males and females, were noted and deserve further study.” (Shak et al, 2009).

*MammaPrint*

MammaPrint a 70-gene profile that classifies breast cancer into Low Risk or High Risk of recurrence, by measuring genes representative of all the pathways of cancer metastases which were selected for their predictive relationship to 10-year recurrence probability (Raman, et al., 2013). MammaPrint is indicated for women who have stage I or II breast cancer, are lymph node positive or negative, are ER-positive or negative and tumor size of less than five centimeters. MammaPrint determines if the patient is a candidate for chemotherapy.

In February 2007, the Food and Drug Administration (FDA) approved MammaPrint (Agendia, Amsterdam), a DNA microarray-based test used to predict whether women with early breast cancer might face the disease again. The test measures the activity of 70 genes, providing information about the likelihood that cancer will recur. It measures each of these genes in a sample of a woman’s breast-cancer tumor and then uses a specific formula to produce a score that determines if the patient is deemed low-risk or high-risk for metastasis. In clinical trials, 1 in 4 women found to be at high risk by MammaPrint had recurrence of their cancer within 5 years. However, there are questions regarding the accuracy of this test. The positive predictive values at 5 and 10 years were 23 % and 29 %, respectively, while the
corresponding negative predictive values were 95 % and 90 %, respectively.

Mammaprint was tested on 307 patients under the age of 61 years who underwent surgery for stage I or stage II breast cancer, and who have tumor size equal to or less than 5 cm, and lymph node-negative. The study found that Mammaprint more than doubled physicians' ability to predict breast cancer recurrence.

Cardoso et al (2016) conducted a study to evaluate the clinical utility of the 70-gene signature test (MammaPrint). The study was excerpted from a phase III randomized trial. In this study, of 6693 enrolled women with early stage breast cancer, women with low clinical and genomic risk did not receive chemotherapy whereas those at high risk did receive chemotherapy. All study subjects had their genomic risk evaluated using MammaPrint. The authors noted that “the primary goal was to assess whether, among patients with high-risk clinical features and a low-risk-gene-expression profile who did not receive chemotherapy, the lower boundary of the 95% confidence interval for the rate of 5-year survival without distant metastasis would be 92% (i.e. the noninferiority boundary, or higher). The number of women found to be at high clinical risk and low genomic risk was 1550. In this group, the 5 year survival rate without distant metastases was 94.7% among those not receiving chemotherapy. The authors concluded that among women with early-stage-breast cancer who were at high clinical risk and low genomic risk for recurrence, the receipt of no chemotherapy on the basis of the 60 gene signature led to a 5-year survival rate without distant metastasis that was 1.5 percentage points lower than the rate with chemotherapy.

A comment by Hudis and Dickler (2016) stated that it can be challenging to convince practitioners that chemotherapy is not needed in an otherwise healthy younger population. They further noted that the primary aim of the study on one study of a 70-gene signature test was to “declare non-inferiority against a predefined benchmark of a 5 year metastasis-free survival rate in just one cohort: patients with a high clinical risk for whom a discordant low genomic risk led to the omission of otherwise standard chemotherapy.” They concluded that although for select patients providers may wish to use the MammaPrint, the actions
they will take as a result of this testing will be variable and may over time change as a result of further study.

The study by Cardoso et al (2016) was a 5-year median follow-up results of the MINDACT trial, which is to follow subjects for 10 years. The authors noted that follow-up is ongoing to determine whether their findings remain valid for longer-term outcome. These investigators noted that “In the critical group of patients at high clinical risk and low genomic risk, the use of adjuvant chemotherapy led to a trend toward a higher rate of the 5-year outcome than that with no chemotherapy, which included a rate of survival without distant metastasis that was 1.5 percentage points higher, a rate of disease-free survival that was 2.8 percentage points higher, and a rate of overall survival that was 1.4 percentage points higher with chemotherapy than with no chemotherapy in the intention-to-treat population and a rate of survival without distant metastasis that was 1.9 percentage points higher, a rate of disease-free survival that was 3 percentage points higher, and a rate of overall survival that was 1.5 percentage points higher with chemotherapy than with no chemotherapy in the per-protocol population. The study was not powered to assess the statistical significance of these differences.

Some 50% of the study patients were defined as being at low clinical risk. In this group, we did not find any meaningful difference in the 5-year rate of survival without distant metastasis between patients at high genomic risk who received chemotherapy and those who did not receive chemotherapy. On the basis of these data, the results for the 70-gene signature do not provide evidence for making recommendations regarding chemotherapy for patients at low clinical risk”.

In an editorial that accompanied the afore-mentioned study, Hudis and Dickler (2016) stated that “a difference of 1.5 percentage points, if real, might mean more to one patient than to another. Thus, the stated difference does not precisely exclude a benefit that clinicians and patients might find meaningful. An adequately powered randomization or a higher threshold for 5-year metastasis-free survival might have provided a more convincing result but would have raised other major challenges for the investigators”.

A focused update by the American Society for Clinical Oncology (ASCO) (Kopp, et al., 2017) states that if a patient has hormone receptor–positive, human epidermal growth factor receptor 2
(HER2)-negative, node-negative breast cancer, the MammaPrint assay may be used in those with high clinical risk to inform decisions on withholding adjuvant systemic chemotherapy due to its ability to identify a good-prognosis population with potentially limited chemotherapy benefit. The guidelines state that, if a patient has hormone receptor–positive, HER2-negative, node-positive breast cancer, the MammaPrint assay may be used in patients with one to three positive nodes and a high clinical risk to inform decisions on withholding adjuvant systemic chemotherapy. However, such patients should be informed that a benefit from chemotherapy cannot be excluded, particularly in patients with greater than one involved lymph node. The guideline update was based upon an assessment of data on clinical utility from the MINDACT trial plus other published literature.

**BluePrint**

Molecular subtyping profile or BluePrint is proposed for the evaluation of an individual’s prognosis when diagnosed with breast cancer. The multigene profile classifies breast cancer into basal type, luminal type and ERBB type (HER2/neu positive) molecular subclasses to stratify an individual's risk to purportedly assist with treatment decisions.

Agendia BluePrint has an 80-gene profile that classifies breast cancer into molecular subtypes (Raman, et al., 2013). The profile separates tumors into Basal-type, Luminal-type and ERBB2-type subgroups by measuring the functionality of downstream genes for each of these molecular pathways to inform the physician of the potential effect of adjuvant therapy.

Krijgsman et al (2012) noted that classification of breast cancer into molecular subtypes maybe important for the proper selection of therapy, as tumors with seemingly similar histopathological features can have strikingly different clinical outcomes. Herein, these researchers reported the development of a molecular subtyping profile (BluePrint), which enables rationalization in patient selection for either chemotherapy or endocrine therapy prescription. An 80-Gene Molecular Subtyping Profile (BluePrint) was developed using 200 breast cancer patient
specimens and confirmed on 4 independent validation cohorts (n = 784). Additionally, the profile was tested as a predictor of chemotherapy response in 133 breast cancer patients, treated with T/FAC neoadjuvant chemotherapy. BluePrint classification of a patient cohort that was treated with neoadjuvant chemotherapy (n = 133) shows improved distribution of pathological Complete Response (pCR), among molecular subgroups compared with local pathology: 56 % of the patients had a pCR in the Basal-type subgroup, 3 % in the MammaPrint low-risk, luminal-type subgroup, 11 % in the MammaPrint high-risk, luminal-type subgroup, and 50 % in the HER2-type subgroup. The group of genes identifying luminal-type breast cancer is highly enriched for genes having an Estrogen Receptor binding site proximal to the promoter-region, suggesting that these genes are direct targets of the Estrogen Receptor. Implementation of this profile may improve the clinical management of breast cancer patients, by enabling the selection of patients who are most likely to benefit from either chemotherapy or from endocrine therapy.

An assessment by the National Institute for Health Research (Ward, et al., 2013) found the evidence for Blueprint was limited. Because of the limited available data identified for this test, the NIHR was unable to draw firm conclusions about its analytical validity, clinical validity (prognostic ability) and clinical utility. The report stated that further evidence on the prognostic and predictive ability of this test was required.

A report by the Belgian Healthcare Knowledge Centre (KCE) (San Miguel, et al., 2015) found that limited evidence for the prognostic ability (clinical validity) of BluePrint. The KCE found insufficient evidence on the impact of BluePrint on clinical management (clinical utility).

Furthermore, there is no information regarding BluePrint/molecular subtyping from NCCN’s clinical practice guideline on “Breast cancer” (Version 2.2013).
TargetPrint®, ER/PR/HER2 Expression Assay (Agendia) is a microarray-based gene expression test which offers a quantitative assessment of the patient’s level of estrogen receptor (ER), progesterone receptor (PR) and HER2/neu overexpression within her breast cancer (Raman, et al., 2013). TargetPrint is offered in conjunction with MammaPrint to provide the physician an even more complete basis for treatment decisions. TargetPrint delivers an added benefit to the diagnostic process. Immunohistochemistry provides a semi-quantitative positive or negative result, whereas the gene expression result provided by TargetPrint allows physicians to integrate the absolute level of ER, PR and HER2 gene expression into treatment planning. TargetPrint determines if the patient is a candidate for hormonal therapy.

TargetPrint is a microarray-based gene expression test which offers a quantitative assessment of the patient’s level of estrogen receptor (ER), progesterone receptor (PR) and HER2/neu overexpression in breast cancer. The manufacturer states that TargetPrint is offered in conjunction with MammaPrint gene expression profiling to provide the physician an even more complete basis for treatment decisions. The manufacturer states that, as compared to Immunohistochemistry (IHC), TargetPrint provides additional information. Whereas IHC provides a semi-quantitative positive or negative result, the gene expression result provided by TargetPrint provides data on the absolute level of ER, PR and HER2 gene expression. Published information on the TargetPrint is limited to studies examining its correlation with measurements of ER, PR, and HER2 receptors (Gunven et al, 2011; Gevensleben et al, 2010; Roepman et al, 2009). There is a lack of evidence from published prospective clinical studies that demonstrates that quantification of ER, PR, and HER2 gene expression by TargetPrint alters management such that clinical outcomes are improved.

Symphony

Symphony (Agendia) provides complete tumor profiling and is used to support therapeutic choices for breast cancer (Raman, 2013). SYMPHONY includes four assays to support breast cancer
treatment decisions: MammaPrint® determines the risk of recurrence. BluePrint™ determines molecular subtypes and TargetPrint® determines estrogen receptor (ER), progesterone receptor (PR), and HER2 status. TheraPrint™ identifies alternative types of therapy for metastatic disease. SYMPHONY provides genomic information assisting with therapeutic decisions even for cases that have been otherwise classified as indeterminate, such as grade 2, small tumors, HER2 and/or lymph node positive. MammaPrint® determines if the patient is a candidate for chemotherapy. TargetPrint® determines if the patient is a candidate for hormonal therapy. BluePrint® provides information on the sub-classification of the tumor which guides the choice of therapies and combinations of therapies. TheraPrint® identifies alternative types of therapy for metastatic disease.

Rotterdam Signature 76-Gene Panel

The Rotterdam Signature test (Veridex) is a 76-gene expression assay (Raman, 2013). Sixty genes are intended to evaluate estrogen-receptor positive samples and 16 genes to evaluate estrogen-receptor negative samples. In a validation study that tested the signature on samples from 148 women, 50 fell into the low-risk group and 98 into the high-risk group. The test had 88% specificity and 39% sensitivity for the low-risk group, with a hazard ratio for distant relapse within 5 years of 5.74 comparing the high-risk group to the low-risk group. The Rotterdam Signature identifies women at high and low risk of disease recurrence.

The Rotterdam Signature 76-gene panel (Veridex, LLC) is a multivariate index assay that is intended to assist in assessing a patient’s risk of systemic recurrence of cancer following successful initial treatment of localized node-negative breast cancer with surgery and tamoxifen alone. This multigene assay is intended for use in lymph-node negative breast cancer patients. The Rotterdam Signature panel uses microarray processing to measure cellular concentrations of mRNA in fresh tissue samples. The Rotterdam Signature panel uses the Human Genome U133a GeneChip (Affymetrix, Inc.) to identify patients that have gene expression signatures associated with either a low or high risk of
developing metastatic disease. A multicenter study investigated the ability of the Rotterdam 76-gene signature to identify patients at risk of distant metastases within 5 and 10 years of first diagnosis, using frozen tissue samples from 180 patients with node-negative breast cancer who had not received systemic chemotherapy (Foekens, et al., 2006). The Rotterdam 76-gene signature correctly identified 27 out of 30 cases of relapse within 5 years (90% sensitivity) and 75 out of 150 patients who did not relapse (50% specificity). An earlier summary of the same study (Foekens, et al., 2005) reported a hazard ratio for distant metastasis-free survival comparing favorable versus unfavorable signature = 7.41 (95% confidence interval 2.63-20.9); p = 8.5 x 10^-6). The hazard ratio of overall survival comparing favorable versus unfavorable signature = 5.45 (95% confidence interval 1.62-18.3); p = .002. There are no published studies that have assessed the clinical utility of the Rotterdam 76-gene signature by monitoring the long-term outcomes of the patients selected and not selected for chemotherapy on the basis of assay results.

**Breast Cancer Gene Expression Ratio / Breast Cancer Index**

The Breast Cancer Gene Expression Ratio (HOXB13:IL17BR, also known as H/I) (AviaraDx, Inc., Carlsbad, CA) is intended to predict the risk of disease recurrence in women with estrogen receptor (ER)-positive, lymph node-negative breast cancer. The Breast Cancer Gene Expression Ratio is based on the ratio of the expression of two genes: the homeobox gene-B13 (HOXB13) and the interleukin-17B receptor gene (IL17BR). In breast cancers that are more likely to recur, the HOXB13 gene tends to be over-expressed, while the IL-17BR gene tends to be under-expressed.

Ma, et al. (2004) reported on the early validation of the HOXB13:IL17BR gene expression ratio. The investigators generated gene expression profiles of hormone receptor-positive primary breast cancers in a set of 60 patients treated with adjuvant tamoxifen monotherapy. An expression signature predictive of disease-free survival was reduced to a two-gene ratio, HOXB13 versus IL17BR, which outperformed existing biomarkers. The investigators concluded that ectopic expression of HOXB13 in MCF10A breast epithelial cells enhances
motility and invasion in vitro, and its expression is increased in both preinvasive and invasive primary breast cancer. The investigators suggested that HOXB13:IL17BR expression ratio may be useful for identifying patients appropriate for alternative therapeutic regimens in early-stage breast cancer.

In an 852-patient retrospective study, Ma, et al (2006) found that the HOXB13:IL17BR ratio (H:I expression ratio) independently predicted breast cancer recurrence in patients with ER-positive, lymph-node negative cancer. The H:I expression ratio was found to be predictive in patients who received tamoxifen therapy as well as in those who did not. Expression of HOXB13, IL17BR, CHDH, estrogen receptor (ER) and progesterone receptor (PR) were quantified by real-time polymerase chain reaction (PCR) in 852 formalin-fixed, paraffin-embedded primary breast cancers from 566 untreated and 286 tamoxifen-treated breast cancer patients. Gene expression and clinical variables were analyzed for association with relapse-free survival (RFS) by Cox proportional hazards regression models. The investigators reported that, in the entire cohort, expression of HOXB13 was associated with shorter RFS (p = .008), and expression of IL17BR and CHDH was associated with longer RFS (p < 0.0001 for IL17BR and p = 0.0002 for CHDH). In ER-positive patients, the HOXB13:IL17BR index predicted clinical outcome independently of treatment, but more strongly in node-negative patients. In multivariate analysis of the ER-positive node-negative subgroup including age, PR status, tumor size, S phase fraction, and tamoxifen treatment, the two-gene index remained a significant predictor of RFS (hazard ratio [HR] = 3.9; 95 % CI:1.5 to 10.3; p = .007).

The value of the Breast Cancer Gene Expression Ratio was also evaluated in a study by Goetz et al (2006). That study found that a high H:I expression ratio is associated with an increased rate of relapse and mortality in ER-positive, lymph node-negative cancer patients treated with surgery and tamoxifen. Goetz et al (2006) examined the association between the ratio of the HOXB13 to IL17BR expression and the clinical outcomes of relapse and survival in women with ER-positive breast cancer enrolled onto a North Central Cancer Treatment Group adjuvant tamoxifen trial (NCCTG 89-30-52). Tumor blocks were obtained from 211 of 256
eligible patients, and quantitative reverse transcription-PCR profiles for HOXB13 and IL-17BR were obtained from 206 patients. In the node-positive cohort (n = 86), the HOXB13/IL-17BR ratio was not associated with relapse or survival. In contrast, in the node-negative cohort (n = 130), a high HOXB13/IL-17BR ratio was associated with significantly worse RFS [HR, 1.98; p = 0.031], disease-free survival (DFS) (HR, 2.03; p = 0.015), and OS (HR, 2.4; p = 0.014), independent of standard prognostic markers.

The Blue Cross and Blue Shield Association Technology Evaluation Center (BCBSA, 2007) announced that its Medical Advisory Panel (MAP) concluded that the use of the Breast Cancer Gene Expression Ratio gene expression profiling does not meet the TEC criteria.

The Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group (2009) found insufficient evidence to make a recommendation for or against the use of the H:I ratio test to improve outcomes in defined populations of women with breast cancer. EGAPP concluded that the evidence is insufficient to assess the balance of benefits and harms of the proposed uses of this test. The EWG encouraged further development and evaluation of these technologies.

In a systematic review on gene expression profiling assays in early-stage breast cancer, Marchionni, et al. (2008) summarized evidence on the validity and utility of 3 gene expression-based prognostic breast cancer tests: Oncotype Dx, MammaPrint, and H/I. The authors concluded that gene expression technologies show great promise to improve predictions of prognosis and treatment benefit for women with early-stage breast cancer. However, more information is needed on the extent of improvement in prediction, characteristics of women in whom the tests should be used, and how best to incorporate test results into decision making about breast cancer treatment.

Guidelines from the American Society for Clinical Oncology (Harris, et al., 2007) found that, in newly diagnosed patients with node-negative, estrogen-receptor positive breast cancer, the
Oncotype Dx assay can be used to predict the risk of recurrence in patients treated with tamoxifen. The ASCO guidelines concluded that Oncotype Dx may be used to identify patients who are predicted to obtain the most therapeutic benefit from adjuvant tamoxifen and may not require adjuvant chemotherapy. The ASCO guidelines found, in addition, that patients with high recurrence scores appear to achieve relatively more benefit from adjuvant chemotherapy than from tamoxifen. ASCO found that there are insufficient data at present to comment on whether these conclusions generalize to hormonal therapies other than tamoxifen, or whether this assay applies to other chemotherapy regimens. Guidelines from the American Society for Clinical Oncology (Harris, et al., 2007) concluded that the precise clinical utility and appropriate application for other multiparameter assays, such as the MammaPrint assay, the Rotterdam Signature, and the Breast Cancer Gene Expression Ratio are under investigation. ASCO also found insufficient data to recommend use of proteomic patterns for management of patients with breast cancer.

Sgori, et al. (2013) found that, in the absence of extended letrozole therapy, high H/I identifies a subgroup of ER-positive patients disease-free after 5 years of tamoxifen who are at risk for late recurrence. The investigators also found that, when extended endocrine therapy with letrozole is prescribed, high H/I predicts benefit from therapy and a decreased probability of late disease recurrence. Sgori, et al. conducted a prospective-retrospective, nested case-control design of 83 recurrences matched to 166 nonrecurrences from letrozole- and placebo-treated patients within MA.17 trial. Expression of H/I within primary tumors was determined by reverse-transcription polymerase chain reaction with a prespecified cutpoint. The investigators determined the predictive ability of H/I for ascertaining benefit from letrozole using multivariable conditional logistic regression including standard clinicopathological factors as covariates. All statistical tests were two-sided. The investigators reported that high H/I was statistically significantly associated with a decrease in late recurrence in patients receiving extended letrozole therapy (odds ratio [OR] = 0.35; 95% confidence interval [CI] = 0.16 to 0.75; P = .007). In an adjusted model with standard clinicopathological
factors, high H/I remained statistically significantly associated with patient benefit from letrozole (OR = 0.33; 95% CI = 0.15 to 0.73; P = .006). Reduction in the absolute risk of recurrence at 5 years was 16.5% for patients with high H/I (P = .007). The interaction between H/I and letrozole treatment was statistically significant (P = .03).

BioTheranostics Breast Cancer Index (BCI) is a prognostic biomarker that provides quantitative assessment of the likelihood of distant recurrence in patients diagnosed with estrogen receptor-positive, lymph node-negative breast cancer (Raman, et al., 2013). In development and validation studies, BCI stratified about 50% of tamoxifen treated ER+, node-negative breast cancer patients into a low risk group for 10-year distant recurrence. BCI is a molecular assay developed from the combination of two indices: HOXB13:IL17BR and five cell cycle-associate gene index (BUB1B, CENPA, NEK2, RACGAP1, RRM2) that assesses tumor grade. The test is performed on a formalin-fixed, paraffin-embedded (FFPE) tissue block.

Ma, et al. (2008) reported on the development and early validation of a five-gene reverse transcription PCR assay for molecular grade index (MGI) that has subsequently been incorporated into BCI and is suitable for analyzing routine formalin-fixed paraffin-embedded clinical samples. The investigators found that the combination of MGI and HOXB13:IL17BR outperformed either alone and identifies a subgroup (approximately 30%) of early stage estrogen receptor-positive breast cancer patients with very poor outcome despite endocrine therapy. From their previously published list of genes whose expression correlates with both tumor grade and tumor stage progression, the investigators selected five cell cycle-related genes to build MGI and evaluated MGI in two publicly available microarray data sets totaling 410 patients. Using two additional cohorts (n =323), the investigators developed a real-time reverse transcription PCR assay for MGI, validated its prognostic utility, and examined its interaction with HOXB13:IL17BR. The investigators reported that MGI performed consistently as a strong prognostic factor and was comparable with a more complex 97-gene genomic grade index in multiple data sets. In
patients treated with endocrine therapy, MGI and HOXB13:IL17BR modified each other's prognostic performance. High MGI was associated with significantly worse outcome only in combination with high HOXB13:IL17BR, and likewise, high HOXB13:IL17BR was significantly associated with poor outcome only in combination with high MGI.

Jerevall, et al. (2011) reported on the development of the Breast Cancer Index, a dichotomous index combining two gene expression assays, HOXB13:IL17BR (H:I) and molecular grade index (MGI), to assess risk of recurrence in breast cancer patients. The study objective was to demonstrate the prognostic utility of the combined index in early-stage breast cancer. In a blinded retrospective analysis of 588 ER-positive tamoxifen-treated and untreated breast cancer patients from the randomized prospective Stockholm trial which was conducted during 1976 to 1990, H:I and MGI were measured using real-time RT-PCR. Association with patient outcome was evaluated by Kaplan-Meier analysis and Cox proportional hazard regression. A continuous risk index was developed using Cox modelling. The investigators found that the dichotomous H:I+MGI was significantly associated with distant recurrence and breast cancer death. The greater than 50% of tamoxifen-treated patients categorized as low-risk had less than 3% 10-year distant recurrence risk. A continuous risk model (Breast Cancer Index (BCI)) was developed with the tamoxifen-treated group and the prognostic performance tested in the untreated group was 53% of patients categorized as low risk with an 8.3% 10-year distant recurrence risk.

Jankowitz, et al. (2011) reported on a study to validate the prognostic performance of BCI in estrogen-receptor positive, lymph node negative breast cancer patients. The investigators found that, in this characteristically low-risk cohort, BCI classified high versus low-risk groups with about a five-fold difference in 10-year risk of distant recurrence and breast cancer-specific death. The investigators identified tumor samples from 265 estrogen-receptor positive, lymph-node negative tamoxifen-treated patients from a single academic institution's cancer research registry. They performed the BCI assay and assigned scores based on a predetermined risk model. The investigators assessed risk by
BCI and Adjuvant Online! (AO) and correlated these to clinical outcomes in the patient cohort. The investigators found that BCI was a significant predictor of outcome in this cohort of estrogen-receptor positive, lymph-node negative patients (median age: 56-y; median follow-up: 10.3-y), treated with adjuvant tamoxifen alone or tamoxifen with chemotherapy (32%). BCI categorized 55%, 21%, and 24% of patients as low, intermediate and high-risk, respectively. The 10-year rates of distant recurrence were 6.6%, 12.1% and 31.9% and of breast cancer-specific mortality were 3.8%, 3.6% and 22.1% in low, intermediate, and high-risk groups, respectively. In a multivariate analysis including clinicopathological factors, BCI was a significant predictor of distant recurrence (HR for 5-unit increase = 5.32 [CI 2.18-13.01; P = 0.0002]) and breast cancer-specific mortality (HR for a 5-unit increase = 9.60 [CI 3.20-28.80; P < 0.0001]). AO was significantly associated with risk of recurrence. In a separate multivariate analysis, both BCI and AO were significantly predictive of outcome. In a time-dependent (10-year) ROC curve accuracy analysis of recurrence risk, the addition of BCI and AO increased predictive accuracy in all patients from 66% (AO only) to 76% (AO+BCI) and in tamoxifen-only treated patients from 65% to 81%. The authors concluded that BCI and AO are independent predictors with BCI having additive utility beyond standard of care parameters that are encompassed in AO. The authors acknowledge that this study is limited by the fact that it was a retrospective, single-institution study and that results may have been biased on the basis of specimen availability and patterns of referral to the tertiary academic center.

Mathieu, et al. (2012) assessed the performance of BCI to predict chemosensitivity based on pathological complete response (pCR) and breast conservation surgery (BCS). The authors performed the BCI assay on tumor samples from 150 breast cancer patients from a single institution treated with neoadjuvant chemotherapy. The authors used logistical regression and c-index to assess predictive strength and additive accuracy of BCI beyond clinicopathologic factors. BCI classified 42% of patients as low, 35% as intermediate and 23% as high risk. Low BCI risk group had 98.4% negative predictive value (NPV) for pCR and 86% NPV for BCS. High versus low BCI group had a 34 and 5.8 greater
likelihood of achieving pCR and BCS, respectively (P=0.0055; P=0.0022). BCI increased c-index for pCR (0.875-0.924; p=0.017) and BCS prediction (0.788-0.843; p=0.027) beyond clinicopathologic factors. The authors concluded that BCI significantly predicted pCR and BCS beyond clinicopathologic factors. High NPVs indicate that BCI could be a useful tool to identify breast cancer patients who are not eligible for neoadjuvant chemotherapy. The authors concluded that "these results suggest that BCI could be used to assess both chemosensitivity and eligibility for BCS." The authors stated that an important limitation of this study is that, in this retrospective analysis, patients were not selected based on ER or HER2 expression for the indications of neoadjuvant chemotherapy. The authors explained that this could have increased the predictive strength of BCI given that this biomarker was initially developed and validated in ER + node-negative patients.

Zhang, et al. (2013) examined the prognostic performance of BCI for prediction of early (0-5 years) and late (more than 5 years) risk of distant recurrence in patients with estrogen receptor-positive (ER+), lymph node-negative (LN-) tumors. The BCI model was validated by retrospective analyses of tumor samples from tamoxifen-treated patients from a randomized prospective trial (Stockholm TAM, n = 317) and a multi-institutional cohort (n = 358). Within the Stockholm TAM cohort, BCI risk groups stratified the majority (approximately 65%) of patients as low risk with less than 3% distant recurrence rate for 0 to 5 years and 5 to 10 years. In the multi-institutional cohort, which had larger tumors, 55% of patients were classified as BCI low risk with less than 5% distant recurrence rate for 0 to 5 years and 5 to 10 years. Zhang and colleagues found that, for both cohorts, continuous BCI was the most significant prognostic factor beyond standard clinicopathologic factors for 0 to 5 years and more than five years. The authors concluded that the prognostic sustainability of BCI to assess early- and late-distant recurrence risk at diagnosis has clinical use for decisions of chemotherapy at diagnosis and for decisions for extended adjuvant endocrine therapy beyond five years.

Sgori, et al. (2013) compared the prognostic ability of the BCI
assay, the Oncotype DX Breast, and IHC4 for both early and late recurrence in patients with estrogen-receptor-positive, node-negative (N0) disease who took part in the Arimidex, Tamoxifen, Alone or in Combination (ATAC) clinical trial. In this prospective comparison study, Sgori and colleagues obtained archival tumor blocks from the TransATAC tissue bank from all postmenopausal patients with estrogen-receptor-positive breast cancer from whom the Oncotype DX and IHC4 values had already been derived. The investigators did BCI analysis in matched samples with sufficient residual RNA using two BCI models -- cubic (BCI-C) and linear (BCI-L)-using previously validated cutoffs. The prospectively-defined primary study objective was to evaluate overall (0–10y) prognostic performance of the BCI-C model for DR in ER+ N0 patients. Secondary objectives were: 1) assessment of the prognostic performance of the BCI-L model and its components, H/I and MGI, for overall (0–10y), early (0–5y) and late (5–10y) DR; 2) comparative performance of BCI-L versus the Oncotype DX RS and IHC4. To assess the ability of the biomarkers to predict recurrence beyond standard clinicopathological variables, the investigators calculated the change in the likelihood-ratio from Cox proportional hazards models. Suitable tissue was available from 665 patients with estrogen-receptor-positive, N0 breast cancer for BCI analysis. The primary analysis showed significant differences in risk of distant recurrence over 10 years in the categorical BCI-C risk groups (p<0.0001) with 6.8% (95% CI 4.4-10.0) of patients in the low-risk group, 17.3% (12.0-24.7) in the intermediate group, and 22.2% (15.3-31.5) in the high-risk group having distant recurrence. BCI-C analyzed as a continuous variable was not significantly associated with overall (0–10y) risk of DR when adjusted for CTS (inter-quartile HR=1.39; 95% CI, 0.99 to 3.70; LR-Δχ²=3.70; P=0.054). Comparison of the prognostic performance of BCI-L to BCI-C indicated that unlike BCI-C, BCI-L was a significant predictor of risk of recurrence as a continuous variable, and the HR after adjustment with CTS was 2.19 versus 4.86 between high- and low-risk groups for BCI-C and BCI-L, respectively. Thus, all subsequent analyses were performed utilizing BCI-L. The secondary analysis showed that BCI-L was a much stronger predictor for overall (0-10 year) distant recurrence compared with BCI-C (interquartile HR 2.30 [95% CI 1.62-3.27]; likelihood ratio (LR)-Δχ²=22.69; p<0.0001). When compared
with BCI-L, the Oncotype Dx breast score was less predictive (HR 1.48 [95% CI 1.22-1.78]; LR-\(\Delta\chi^2\)=13.68; p=0.0002) and IHC4 was similar (HR 1.69 [95% CI 1.51-2.56]; LR-\(\Delta\chi^2\)=22.83; p<0.0001). All further analyses were done with the BCI-L model. In a multivariable analysis, all assays had significant prognostic ability for early distant recurrence (BCI-L HR 2.77 [95% CI 1.63-4.70], LR-\(\Delta\chi^2\)=15.42, p<0.0001; Oncotype Dx Breast score HR 1.80 [1.42-2.29], LR-\(\Delta\chi^2\)=18.48, p<0.0001; IHC4 HR 2.90 [2.01-4.18], LR-\(\Delta\chi^2\)=29.14, p<0.0001); however, only BCI-L was significant for late distant recurrence (BCI-L HR 1.95 [95% CI 1.22-3.14], LR-\(\Delta\chi^2\)=7.97, p=0.0048; 21-gene recurrence score HR 1.13 [0.82-1.56], LR-\(\Delta\chi^2\)=0.48, p=0.47; IHC4 HR 1.30 [0.88-1.94], LR-\(\Delta\chi^2\)=1.59, p=0.20). The authors concluded that BCI-L was the only significant prognostic test for risk of both early and late distant recurrence and identified two risk populations for each timeframe. BCI-L could help to identify patients at high risk for late distant recurrence who might benefit from extended endocrine or other therapy. An important limitation is that the evaluation of BCI-L was a secondary objective of this study; the primary objective was evaluation of BCI-C.

An editorial (Ignatiadis, 2013) accompanying the study by Sgroi, et al. stated that the BCI test is “ready for prime time” in treatment decision making for post-menopausal, estrogen-receptor positive women who have undergone 5 years of hormonal therapy. The editorial noted that there are other molecular diagnostic assays that also have been shown to predict late recurrence. For support, the editorial cited a study by Sestak, et al. (2013), which found that, in the last follow-up phase, Clinical Treatment Score (CTS) added most prognostic information for distant recurrence in years 5 to 10 for breast cancer patients in the ATAC trial. Sestak, et al. reported that, in a multivariate model that incorporated CTS, PAM50 provided the strongest additional prognostic factor in the 5 to 10 year followup phase, followed by BCI, and with IHC4 and RS adding the least prognostic information.

A manufacturer funded study (Gustavsen, et al., 2014) reported on a model that found BCI to be cost saving from a third-party payer perspective, based upon assumptions about the impact of
BCI on adjuvant chemotherapy use, extended endocrine therapy use, and endocrine therapy compliance. The authors developed two economic models to project the cost-effectiveness of BCI in a hypothetical population of patients with estrogen-receptor positive, lymph-node negative breast cancer compared with standard clinicopathologic diagnostic modalities. The authors modeled costs associated with adjuvant chemotherapy, toxicity, followup, endocrine therapy, and recurrence over 10 years. The models examined cost utility compared with standard practice when used at diagnosis and in patients disease-free at 5 years post diagnosis. The authors reported that use of BCI was projected to be cost saving in both models. In the newly diagnosed population, net cost savings were $3803 per patient tested. In the 5 years post diagnosis population, BCI was projected to yield a net cost savings of $1803 per patient tested. The authors reported that sensitivity analyses demonstrated that BCI was cost saving across a wide range of clinically relevant input assumptions.

Preliminary data suggest that molecular approaches including gene expression platforms such as BCI may add to classical clinical parameters including tumor size and node status at diagnosis, but further research is needed (Smith, et al., 2014; Bianchini & Gianni, 2013; Ignatiadis and Sotiriou, 2013). The clinical utility of BCI and other molecular diagnostics in predicting late recurrence has yet to be established (Foukakis and Bergh, 2015). It also remains to be established which of several molecular diagnostic tests in development are the most appropriate for detecting late recurrence (Sestak & Kuzick, 2015).

An assessment by the National Institute for Health Research (Ward, et al., 2013) found that, based on the limited available data, no firm conclusions can be drawn about the analytical validity, clinical validity (prognostic ability) and clinical utility of the Breast Cancer Index. The assessment stated that further evidence on the prognostic and predictive ability of this test is required. An assessment by IETS (2013) and a consensus statement (Azim, et al., 2013) reached similar conclusions.
An assessment by the BlueCross BlueShield Association (2015) concluded that the evidence is insufficient to permit conclusions about the Breast Cancer Index on health outcomes. Although evidence supports the association of risk classes defined by the Breast Cancer Index and recurrence and survival outcomes, it remains to be shown whether the Breast Cancer Index adds incremental prognostic information to standard clinical risk classifiers.

An assessment by the Belgian Healthcare Knowledge Centre (KCE) (San Miguel, et al., 2015) found that the evidence for the H/I ratio assay is limited to studies supporting the prognostic ability (clinical validity) of the test. They found insufficient evidence for the impact of the H/I ratio assay on clinical management (clinical utility).

A review published in the ASCO Educational Book (Smith, et al., 2014) reviewed the BCI and other currently available molecular diagnostics for selecting and determining the optimal duration of endocrine adjuvant therapy in women with early stage estrogen receptor positive breast cancer: "Further research into applying molecular features and gene expression scores to standard clinico-pathologic criteria for tailoring extended endocrine therapy is now a high priority.... An important research challenge is now to identify which patients are likely to benefit from this type of long-term therapy. Preliminary data suggest that molecular approaches including gene expression platforms such as ROR may add to classical clinical parameters including tumor size and node status at diagnosis."

A Palmetto Medicare Local Coverage Determination (LCD) allows coverage of the Breast Cancer Index in certain post-menopausal women with estrogen-receptor positive breast cancer, reasoning that the data defined benefit of the BCI test appears to be when a woman is having significant side effects or has other concerns regarding adjuvant tamoxifen therapy and is opposed to taking more than 5 years of tamoxifen or starting on an AI (letrozole) after tamoxifen (CMS, 2014). The LCD noted, however, that, there is an increase in recurrence risk with increasing BCI score such that, "at the 95% confidence interval
(CI), the risk in some individuals categorized in the BCI-low group could be as high as 20%. Due to the data complexity, there is a significant possibility that a physician might consider all BCI-L patients at negligible risk, and thus not consider extended hormone therapy and consequently lead women from the NCCN recommended interventions. Given the low toxicity and low cost of extended therapy, the false sense of security could deny many women from lifesaving therapy."

There is a lack of consensus among guidelines regarding the value of molecular assays in determining whether longer durations of adjuvant endocrine therapy beyond 5 years are clinically indicated. Guidelines from the American Society for Clinical Oncology (Burstein, et al., 2014) on adjuvant endocrine therapy for hormone-receptor positive breast cancer state: "Well-established clinical factors including tumor size; nodal status; ER, PgR, and HER2 biomarkers; and molecular diagnostic assays serve as prognostic factors for breast cancer recurrence. However, there are no robust specific clinical or biomarker measures that selectively predict early versus late recurrence, nor predict whether tamoxifen or AI therapy would be appropriate treatment, nor determine whether longer durations of adjuvant endocrine therapy are clinically indicated." The National Comprehensive Cancer Network guidelines for breast cancer version 2, 2015 states: "Multiple other multi-gene mor multi-gene expression assay systems have been developed. These systems are generally based upon small, retrospective studies, and the Panel believes that none are currently sufficiently validated to warrant inclusion in the guideline." The St. Gallen guideline panel (Coates, et al., 2015) found that Oncotype DX, MammaPrint, PAM-50 ROR score, EndoPredict and the Breast Cancer Index were all considered usefully prognostic for years 1-5, but only the Oncotype Dx commanded a majority in favor of its value in predicting the usefulness of chemotherapy. The Panel agreed that the PAM50 ROR score was clearly prognostic beyond five years, and that the MammaPrint was not prognostic beyond 5 years. The Panel was divided about the prognostic value of the Breast Cancer Index, the Oncotype DX, and EndoPredict in this time period. ESMO guidelines (Senkus, et al., 2013) state: "Molecular signatures for ER-positive breast cancer such as
OncotypeDx, EndoPredict, Breast Cancer Index or for all types of breast cancer (pNO-1) such as MammaPrint and Genomic Grade Index are commercially available, but none of them have proven robust clinical utility so far. In some cases of difficult decision, such as grade 2 ER-positive HER-2 negative and node-negative breast cancer, MammaPrint and Oncotype DX may be used in conjunction with all clinicopathological factors, to help in treatment decision-making.

Guidelines from the American Society for Clinical Oncology (2016) state: "If a patient has ER/PgR-positive, HER2-negative (node-negative) breast cancer, the clinician may use the Breast Cancer Index to guide decisions on adjuvant systemic therapy." This is a moderate strength recommendation based upon intermediate quality evidence. ASCO guidelines recommend use of the Breast Cancer Index to guide decisions on adjuvant systemic therapy in patients with ER/PgR-positive, HER2-negative (node-positive) breast cancer. The guidelines also recommend against the use of the Breast Cancer Index in HER2-positive breast cancer or TN breast cancer. The guidelines also recommended against the use of The Breast Cancer Index to guide decisions on extended endocrine therapy for patients with ER/PgR-positive, HER-2 negative (node-negative) breast cancer who have had 5 years of endocrine therapy without evidence of recurrence.

**Mammostrat**

Mammostrat (Clarient) is a novel test for estimating the risk for recurrence in hormone-receptor positive, early stage breast cancer that is independent of proliferation and grade (Raman, et al., 2013). Five biomarkers are combined with a defined mathematical algorithm resulting in a risk index. Mammostrat is clinically validated and has been studied on more than 4,500 total patients in numerous independent cohorts that include the NSABP B14 and B20 trials. Clinicians and patients are faced with difficult choices as to whether to add toxic adjuvant chemotherapy in addition to standard endocrine treatment. Mammostrat may help clinicians understand the inherent aggressiveness of the tumor and the likelihood of tumor recurrence.
The Mammostrat is a prognostic immunohistochemistry (IHC) test that measures the risk of breast cancer recurrence in post-menopausal, node-negative, estrogen receptor-expressing breast cancer patients who will receive hormonal therapy and are considering adjuvant chemotherapy. The test analyzes five monoclonal antibody biomarkers and applies a diagnostic algorithm to assess whether patients have a high, moderate, or low risk of recurrence after they have had their breast cancer tumor surgically removed and have been treated with tamoxifen.

Bartlett et al (2010) tested the efficacy of the Mammostrat in a mixed population of cases treated in a single center with breast-conserving surgery and long-term follow-up. Tissue microarrays from a consecutive series of 1,812 women managed by wide local excision and post-operative radiotherapy were collected. Of 1,390 cases stained, 197 received no adjuvant hormonal or chemotherapy, 1,044 received tamoxifen only, and 149 received a combination of hormonal therapy and chemotherapy. Median age at diagnosis was 57 years, 71% were post-menopausal, 23.9% were node-positive and median tumor size was 1.5 cm. Samples were stained using triplicate 0.6 mm2 tissue microarray cores, and positivity for p53, HTF9C, CEACAM5, NDRG1 and SLC7A5 was assessed. Each case was assigned a Mammostrat risk score, and distant recurrence-free survival (DRFS), relapse-free survival (RFS) and overall survival (OS) were analyzed by marker positivity and risk score. Increased Mammostrat scores were significantly associated with reduced DRFS, RFS and OS in ER-positive breast cancer (p < 0.00001). In multivariate analyses the risk score was independent of conventional risk factors for DRFS, RFS and OS (p < 0.05). In node-negative, tamoxifen-treated patients, 10-year recurrence rates were 7.6 +/- 1.5% in the low-risk group versus 20.0 +/- 4.4% in the high-risk group. Further, exploratory analyses revealed associations with outcome in both ER-negative and untreated patients. The authors concluded that the Mammostrat can act as an independent prognostic tool for ER-positive, tamoxifen-treated breast cancer and the results of the study revealed a possible association with outcome regardless of node status and ER-negative tumors.

There is insufficient evidence to determine whether the
Mammostrat test is better than conventional risk assessment tools in predicting the recurrence of breast cancer. Furthermore, neither NCCN or ASCO have incorporated the test into their guidelines as a management tool. Guidance from the National Institute for Health and Clinical Excellence (NICE, 2013) states that the Mammostrat is "only recommended 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 ... The tests are not recommended for general use in these people because of uncertainty about their overall clinical benefit and consequently their cost effectiveness."

An assessment by the Belgian Healthcare Knowledge Centre (KCE) (San Miguel, et al., 2015) found that the evidence for Mammostrat is mainly limited to studies supporting the prognostic ability (clinical validity) of the test. The KCE stated that these studies include a large sample size and appear to be of reasonable quality. The KCE cited one study reporting on clinical utility in terms of the predictive ability of the test by risk group. "However, further evidence is required."

Guidelines from the American Society for Clinical Oncology (2016) state: "If a patient has ER/PgR-positive, HER2-negative (node-positive or node-negative) breast cancer, the clinician should not use the five-protein assay (Mammostrat; Clarent, a GE Healthcare company, Aliso Viejo, CA) to guide decisions on adjuvant systemic therapy." This is a moderate strength recommendation based upon intermediate-quality evidence. The ASCO guidelines recommend against the use of Mammostrat to guide decisions on adjuvant systemic therapy for patients with HER2-positive or TN breast cancer.

OvaChek

The OvaCheck™ (Correlogic Systems, Inc.) is a proteomic analysis of blood for the early detection of ovarian cancer. A similar test, which involved a different molecular pattern, was the subject of a 2002 study of 216 women with ovarian cancer. That study showed that the proteomic test had a specificity of 100% and a
sensitivity of 95%, with a positive predictive value of 94% (Petricoin, et al., 2002). While this study showed that a proteomic test detected ovarian cancers even where CA-125 levels were normal, this study included only women who had been detected with ovarian cancer by other means. There is inadequate evidence that this test will be effective for screening women with undetected ovarian cancer.

In addition, there is concern, given the low prevalence of ovarian cancer, that this test is not sufficiently specific for use in screening. The National Cancer Institute explains that even an ovarian cancer test with a specificity of 99% means that 1% of those who did not have cancer would test positive, which is “far too high a rate for commercial use” (NCI, 2004). For a rare disease such as ovarian cancer, which has an approximate prevalence of 1 in 2,500 in the general population, a 99% specificity and 100% sensitivity translates into 25 women falsely identified for every one true cancer found.

The OvaCheck™ test employs electrospray ionization (ESI) type of mass spectrometry using highly diluted denatured blood samples. This method differs from a matrix-assisted laser desorption ionization (MALDI) analysis of undiluted native sera samples that was used in the Lancet study and is currently under investigation by the National Cancer Institute and Food and Drug Administration (NCI, 2004). The NCI notes that “[t]he class of molecules analyzed by these two approaches, and thus the molecules that constitute the diagnostic patterns, would be expected to be entirely different.” Neither the NCI nor FDA has been involved in the design or validation of OvaCheck™ methodology.

As the Ovacheck test is performed as a “home-brewed” test by two national laboratories instead of as a commercially available kit, FDA approval of the OvaCheck test may not be required. The Society for Gynecologic Oncologists (SGO, 2004) has reviewed the literature regarding OvaCheck and concluded that “more research is needed to validate the test’s effectiveness before offering it to the public.” Similarly, the American College of Obstetricians and Gynecologists (2004) has stated that "more research is needed to
validate the test's effectiveness before recommending it to the public."

An assessment of the Ovacheck test and other genomic tests for ovarian cancer prepared for the Agency for Healthcare Research and Quality by the Duke Evidence-Based Practice Center (Myers, et al., 2006) reached the following conclusions: "Genomic test sensitivity/specificity estimates are limited by small sample sizes, spectrum bias, and unrealistically large prevalences of ovarian cancer; in particular, estimates of positive predictive values derived from most of the studies are substantially higher than would be expected in most screening or diagnostic settings. We found no evidence relevant to the question of the impact of genomic tests on health outcomes in asymptomatic women. Although there is a relatively large literature on the association of test results and various clinical outcomes, the clinical utility of changing management based on these results has not been evaluated." Specifically regarding Ovacheck and other proteomic tests for ovarian cancer, the assessment found that, "[a]lthough all studies reported good discrimination for the particular protein profile studied, there were several recurrent issues that limit the ability to draw inferences about potential clinical applicability," in particular technical issues with the assays themselves, variations in analytic methods used among studies, and an unrealistically high prevalence of ovarian cancer in the datasets compared to what would be expected in a normal screening population.

_OvaSure_

OvaSure is an ovarian cancer screening test that entails the use of 6 biomarkers (leptin, prolactin, osteopontin, insulin-like growth factor II, macrophage inhibitory factor and CA-125) to assess the presence of early stage ovarian cancer in high-risk women. Visintin et al (2008) characterized and validated the OvaSure for discriminating between disease-free and ovarian cancer patients. These researchers analyzed 362 healthy controls and 156 newly diagnosed ovarian cancer patients. Concentrations of leptin, prolactin, osteopontin, insulin-like growth factor II, macrophage inhibitory factor, and CA-125 were determined using a multiplex, bead-based, immunoassay system. All 6 markers were evaluated
in a training set (181 samples from the control group and 113 samples from ovarian cancer patients) and a test set (181 sample control group and 43 ovarian cancer). Multiplex and ELISA exhibited the same pattern of expression for all the biomarkers. None of the biomarkers by themselves was good enough to differentiate healthy versus cancer cells. However, the combination of the 6 markers provided a better differentiation than CA-125. Four models with less than 2% classification error in training sets all had significant improvement (sensitivity 84% to 98% at specificity 95%) over CA-125 (sensitivity 72% at specificity 95%) in the test set. The chosen model correctly classified 221 out of 224 specimens in the test set, with a classification accuracy of 98.7%. The authors noted that the OvaSure is the first blood biomarker test with a sensitivity of 95.3% and a specificity of 99.4% for the detection of ovarian cancer. Six markers provided a significant improvement over CA-125 alone for ovarian cancer detection. Validation was performed with a blinded cohort. They stated that this novel multiplex platform has the potential for efficient screening in patients who are at high risk for ovarian cancer.

However, the Society of Gynecologic Oncologists (SGO, 2008) released an opinion regarding OvaSure, which stated that additional research is needed before the test should be offered to women outside the context of a research study. Moreover, SGO stated that it will "await the results of further clinical validation of OvaSure with great interest".

Furthermore, according to the FDA's web site, the FDA sent the Laboratory Corporation of America a warning letter stating that it is illegally marketing OvaSure to detect ovarian cancer. According to the FDA warning letter, their review indicates that this product is a device under section 201(h) of the Food, Drug, and Cosmetic Act (FDCA or Act), 21 U.S.C. 321(h), because it is intended for use in the diagnosis of disease or other conditions, or in the cure, treatment, prevention, or mitigation of disease. The Act requires that manufacturers of devices that are not exempt obtain marketing approval or clearance for their products from the FDA before they may offer them for sale. This helps protect the public health by ensuring that new devices are shown to be both safe
and effective or substantially equivalent to other devices already legally marketed in this country for which approval is not required. According to the FDA warning letter, no such determination has been made for OvaSure.

NCCN Guidelines Panel Members (NCCN, 2016) believe that the OvaSure screening test should not be used to detect ovarian cancer. The NCCN guidelines explain that the OvaSure test uses 6 biomarkers, including leptin, prolactin, osteopontin, insulin-like growth factor II, macrophae inhibitory factor, and CA-125.

**Thrombospondin-1**

Thrombospondin-1 (THBS-1), an angiogenesis inhibitor, has been identified as a potential monitoring marker in gynecologic malignancies. In a randomized phase III study on the co-expression of angiogenic markers and their associations with prognosis in advanced epithelial ovarian cancer, Secord, et al. (2007) reported that high THBS-1 may be an independent predictor of worse progression-free and overall survival in women with advanced-stage EOC. However, the authors stated, "A larger prospective study is warranted for validation of these findings."

**Previstage GCC**

Guanylyl Cyclace C (GCC or GUCY2C) (Diagnocure) a gene coding for a protein found in cells, lining the intestine from the duodenum to the rectum (Raman, et al., 2013). It is involved in water transport, crypt morphology and suppression of tumorigenesis. It is not normally found in tissue in other parts of the body, and therefore, GCC detected outside of the intestine, indicates presence of colorectal cancer metastases. Early studies have indicated that the presence of GCC in the blood may be an early indicator of micrometastases that would otherwise escape detection by the current standard methods of monitoring. Earlier detection provides an opportunity for more immediate treatment or surgical intervention to potentially improve patient outcomes and survival rates. This is a diagnostic test for recurrence by identification of micrometastasis in the blood.
Guanyl cyclase C (GCC) is a receptor protein normally expressed in high concentrations on the luminal surface of the gastrointestinal epithelium. Expression of GCC persists on mucosal cells that have undergone malignant transformation. Thus, GCC has potential use as a marker to determine spread of colorectal cancer to lymph nodes. A retrospective study of 21 patients post surgical resection of colorectal cancer found that all 11 of 21 patients who were free of cancer for 5 years or more were negative for GCC in lymph nodes, whereas all 10 of 21 patients whose cancer returned within 3 years of surgery were positive for GCC. However, the value of the GCC marker test in the management of colorectal cancer needs to be evaluated in prospective clinical outcome studies. A large prospective study is currently being conducted to compare standard histological examination of lymph nodes to the GCC marker test.

Previstage™ Guanylyl Cyclase C (GCC or GUCY2C) (Diagnocure) is a gene coding for a protein found in cells, lining the intestine from the duodenum to the rectum (Raman, et al., 2013). It is involved in water transport, crypt morphology and suppression of tumorigenesis. It is not normally found in tissue in other parts of the body, and therefore, GCC detected outside of the intestine, indicates presence of colorectal cancer metastases. GCC mRNA has shown to be highly accurate in detecting the spread and recurrence of colorectal cancer, respectively in lymph nodes and blood, thereby representing a significant improvement over traditional detection methods. Previstage is a predictive test for risk stratification of recurrence and prognostic marker for recurrence.

Thymidylate Synthase

Thymidylate synthase is a DNA synthesis related gene. According to Compton (2008), the prognostic value of this promising and potentially clinically applicable molecular marker has been studied in colorectal cancer. Compton found that the independent influence of this marker on prognosis remains unproven. Compton explained that "[v]ariability in assay methodology, conflicting results from various studies examining the same factor, and the prevalence of multiple small studies that
lack statistically robust, multivariate analyses all contribute to the lack of conclusive data." Compton concluded that before this marker can be incorporated into clinically meaningful prognostic stratification systems, more studies are required using multivariate analysis, well-characterized patient populations, reproducible and current methodology, and standardized reagents.

In a special report on pharmacogenomics of cancer, the BlueCross and BlueShield Association's Technology Evaluation Center (TEC) (2007) described the results of a meta-analysis on thymidylate synthase protein expression and survival in colorectal cancer that stated low thymidylate synthase expression was significantly associated with better survival, but heterogeneity and possible bias prevented firm conclusions.

Guidelines from the American Society for Colon and Rectal Surgeons (2004) stated: "In the future, DNA analysis and the intratumoral expression of specific chemical substances", including thymidylate synthase, "may be used routinely to further assess prognosis or response to therapy." In addition, Shankaran et al (2008) stated in a review on the role of molecular markers in predicting response to therapy in patients with colorectal cancer, "Although to date no molecular characteristics have emerged as consistent predictors of response to therapy, retrospective studies have investigated the role of a variety of biomarkers, including microsatellite instability, loss of heterozygosity of 18q, type II transforming growth factor beta receptor, thymidylate synthase, epidermal growth factor receptor, and Kirsten-ras (KRAS)."

**VEGF**

Tumour angiogenesis is associated with invasiveness and the metastatic potential of various cancers. Vascular endothelial growth factor (VEGF), the most potent and specific angiogenic factor identified to date, regulates normal and pathologic angiogenesis. An evidence report from Cancer Care Ontario (Welch et al, 2008) on the use of the VEGF inhibitor bevacizumab in colorectal cancer explained that the increased expression of
VEGF has been correlated with metastasis, recurrence, and poor prognosis in many cancers, including colorectal cancer. Guidelines from the National Institute for Health and Clinical Excellence (NICE, 2007) explained that bevacizumab (Avastin) is a recombinant humanised monoclonal IgG1 antibody that acts as an angiogenesis inhibitor. It targets the biological activity of VEGF, which stimulates new blood vessel formation in the tumour. However, neither the FDA approved labeling of bevacizumab or evidence-based guidelines recommend measurement of VEGF to diagnose colorectal cancer or to select patients for treatment. In a special report on pharmacogenomics of cancer, the BlueCross and BlueShield Association's Technology Evaluation Center (TEC) (2007) stated that pre-treatment VEGF levels do not appear to be predictive of response to anti-angiogenic therapy.

Shin and colleagues (2013) evaluated inhibitory effects of bevacizumab on VEGF signaling and tumor growth in-vitro and in-vivo, and assessed phosphorylation of VEGF receptor 2 (VEGFR2) and downstream signaling in endothelial cells as pharmacodynamic markers using phospho-flow cytometry. These researchers also validated markers in patients with mCRC treated with bevacizumab-based chemotherapy. In in-vitro studies, bevacizumab inhibited proliferation of human umbilical vein endothelial cells in association with reduced VEGF signaling. Notably, bevacizumab inhibited VEGF-induced phosphorylation of VEGFR-2, Akt, and extra-cellular signal-regulated kinase (ERK). In-vivo, treatment with bevacizumab inhibited growth of xenografted tumors and attenuated VEGF-induced phosphorylation of Akt and ERK. The median percentages of VEGFR2 + pAkt + and VEGFR2 + pERK + cells, determined by phospho-flow cytometry, were approximately 3-fold higher in mCRC patients than in healthy controls. Bevacizumab treatment decreased VEGFR2 + pAkt + cells in 18 of 24 patients on day 3. The authors concluded that bevacizumab combined with chemotherapy decreased the number of VEGFR2 + pAkt + cells, reflecting impaired VEGFR2 signaling. Together, these data suggested that changes in the proportion of circulating VEGFR2 + pAkt + cells may be a potential pharmacodynamic marker of the effectiveness of anti-angiogenic agents, and could prove valuable...
in determining drug dosage and administration schedule.

*ProstatePx*

Donovan et al (2008) from Aureon, the manufacturer of Prostate Px, reported on the development and validation of their systems pathology model for predicting prostate cancer recurrence after prostatectomy. The clinical utility of defining high risk for failure after radical prostatectomy is to decide whether patients require closer follow-up than average or whether adjuvant radiotherapy, hormone therapy, or chemotherapy would be of benefit. In this analysis, the concordance index for the systems pathology approach used by Aureon was 0.83, but was only slightly better than a 10-variable model that used only the usual clinical parameters, with a concordance index of 0.80. The corresponding hazard ratios for clinical failure were 6.37 for the 10-variable clinical model, and 9.11 for the systems pathology approach. In an accompanying editorial, Klein, et al. (2009) questioned the clinical significance of these differences. They noted that "[a]lthough the difference in concordance indices was statistically significant, the question is whether there is sufficient clinical relevance to justify the extra effort, expense, and clinical expertise needed for the systems approach ... In contemporary clinical practice, a patient with a hazard ratio of 6.37 generated by the model using easily derived, routinely reported clinical and pathological parameters is just as likely to be a candidate for closer monitoring or adjuvant therapy than one with a hazard ratio of 9.11 generated by the systems approach".

Sutcliffe et al (2009) provided an evidence-based perspective on the prognostic value of novel markers in localized prostate cancer and identified the best prognostic model including the 3 classical markers and investigated if models incorporating novel markers are better. Eight electronic bibliographic databases were searched. The reference lists of relevant articles were checked and various health services research-related resources consulted via the internet. The search was restricted to publications from 1970 onwards in the English language. Selected studies were assessed, data extracted using a standard template, and quality assessed using an adaptation of published criteria. Because of the
heterogeneity regarding populations, outcomes and study type, meta-analyses were not undertaken and the results are presented in tabulated format with a narrative synthesis of the results. A total of 30 papers met the inclusion criteria, of which 28 reported on prognostic novel markers and 5 on prognostic models. A total of 21 novel markers were identified from the 28 novel marker studies. There was considerable variability in the results reported, the quality of the studies was generally poor and there was a shortage of studies in some categories. The marker with the strongest evidence for its prognostic significance was PSA velocity (or doubling time). There was a particularly strong association between PSA velocity and prostate cancer death in both clinical and pathological models. In the clinical model the hazard ratio for death from prostate cancer was 9.8 (95% CI 2.8 to 34.3, p < 0.001) in men with an annual PSA velocity of more than 2 ng/ml versus an annual PSA velocity of 2 ng/ml or less; similarly, the hazard ratio was 12.8 (95% CI 3.7 to 43.7, p < 0.001) in the pathological model. The quality of the prognostic model studies was adequate and overall better than the quality of the prognostic marker studies. Two issues were poorly dealt with in most or all of the prognostic model studies: (i) inclusion of established markers, and (ii) consideration of the possible biases from study attrition. Given the heterogeneity of the models, they can not be considered comparable. Only 2 models did not include a novel marker, and 1 of these included several demographical and co-morbidity variables to predict all-cause mortality. Only 2 models reported a measure of model performance, the C-statistic, and for neither was it calculated in an external data set. It was not possible to assess whether the models that included novel markers performed better than those without. This review highlighted the poor quality and heterogeneity of studies, which render much of the results inconclusive. It also pinpointed the small proportion of models reported in the literature that are based on patient cohorts with a mean or median follow-up of at least 5 years, thus making long-term predictions unreliable. Prostate-specific antigen velocity, however, stood out in terms of the strength of the evidence supporting its prognostic value and the relatively high hazard ratios. There is great interest in PSA velocity as a monitoring tool for active surveillance but there is as yet no consensus on how it should be
used and, in particular, what threshold should indicate the need for radical treatment.

In an editorial on clinically relevant prognostic markers for prostate cancer, Gelmann and Henshall (2009) stated that "[u]ntil we have sufficiently discriminating markers to inform treatment decisions, the problem of whom to treat will continue to grow exponentially as the number of cases of screening-detected low-risk cancer increases".

**Circulating Tumor Cells (e.g., CellSearch)**

Circulating tumor cell (CTC) test, CellSearch, is a blood test that has been proposed as a method to determine prognosis, evaluate progression and assess treatment response in individuals with metastatic breast, colorectal and prostate cancers. CTC assays were developed to detect cells that break away from tumors and enter the blood stream.

The CellSearch™ Epithelial Cell Kit, along with the CellSpotter™ Analyzer (Veridex, LLC, Warren, NJ) is a device designed to automate the detection and enumeration of circulating tumor cells (CTCs) of epithelial origin (CD45-, EpCAM+, and cytokeratins 8, 18+ and/or 19+) in whole blood in patients with advanced breast cancer (Ellery, et al., 2010; Raman, et al., 2011). It is intended for use in adjunctively monitoring and predicting cancer disease progression and response to therapy.

The CellSearch Epithelial Cell Kit received FDA 510(k) clearance on January 21, 2004. The FDA concluded that the device is substantially equivalent to immunomagnetic circulating cancer cell selection and enumeration systems. These devices consist of biological probes, fluorochromes and other reagents, preservation and preparation devices and semi-automated analytical instruments to select and count circulating cancer cells in a prepared sample of whole blood.

The CellSearch Epithelial Cell Kit quantifies CTCs by marking cancerous cells with tiny, protein-coated magnetic balls in whole blood. These cells are stained with fluorescent markers for
identification and then dispensed into a cartridge for analysis where a strong magnetic field is applied to the mixture causing the magnetically marked cells to move to the cartridge surface. The cartridge is then analyzed by the CellSpotter Analyzer. A medical professional rechecks the CTCs and the CellSpotter Analyzer tallies the final CTC count.

In a prospective, multicenter study, Cristofanilli et al (2004) used the CellSearch System on 177 patients with measurable metastatic breast cancer for levels of CTCs both before the patients started a new line of therapy and at follow-up. The progression of the disease or the response to treatment was determined with the use of standard imaging studies at the participating centers every nine to twelve weeks. Outcomes were assessed according to levels of CTCs at baseline, before the patients started a new therapy. In the first test, patients with 5 or more CTCs per 7.5 ml of blood compared to a group with fewer than 5 CTCs had a shorter median progression-free survival (2.7 months vs. 7.0 months) and shorter overall survival (10.1 months vs. greater than 18 months). At the follow-up visit, approximately three to four weeks after the initiation of therapy, the percentage of patients with more than 5 CTC was reduced from 49 percent to 30 percent, suggesting a benefit from therapy. The difference in progression-free survival between the two groups remained consistent (2.1 months for women with 5 or more CTCs vs. 7 months for women with less than 5 CTCs). Overall, survival in the women with more than 5 CTCs was 8.2 months compared to greater than 18 months in the cohort with less than 5 CTCs. Cristofanilli concluded that the number of CTCs before treatment was an independent predictor of progression-free survival and overall survival in patients with metastatic breast cancer. However, Cristofanilli also concluded that the results may not be valid for patients who do not have measurable disease or for those starting a new regimen of hormone therapy, immunotherapy, or both. He states, “The prognostic implications of an elevated level of circulating tumor cells for patients with metastatic disease who are starting a new treatment may be an opportunity to stratify these patients in investigational studies”. Furthermore, the study did not address whether patients with an elevated number of circulating tumor cells might benefit from
other therapies. Thus, this minimally invasive assay requires further evaluation as a prognostic marker of disease progression and response to therapy.

The clinical application of quantifying CTCs in the peripheral blood of breast cancer patients remains unclear. Published data in the peer-reviewed medical literature are needed to determine how such measurements would guide treatment decisions and whether these decisions would result in beneficial patient outcomes (Kahn, et al., 2004; Abelooff, et al., 2004). An assessment of CellSearch by AETSA (2006) concluded "In the current stage of development of this technology, there is no evidence that it provides any advantage over existing technology for CTC identification or indeed any additional clinical use." Guidelines from the American Society for Clinical Oncology (Harris, et al., 2007) found: "The measurement of circulating tumor cells (CTCs) should not be used to make the diagnosis of breast cancer or to influence any treatment decisions in patients with breast cancer. Similarly, the use of the recently U.S. Food and Drug Administration (FDA)-cleared test for CTC (CellSearch Assay) in patients with metastatic breast cancer cannot be recommended until further validation confirms the clinical value of this test."

An assessment by the Canadian Agency for Drugs and Technologies in Health (CADTH, 2012) found that studies indicate that measurement of CTCs using the CellSearch system could be used as prognostic factors for progression of the disease and the potential treatment of patients with ovarian cancer. No economic studies were identified, therefore the cost-effectiveness of the CellSearch system could not be summarized.

Although studies relate circulating tumor cells to prognostic indicators (see, e.g., Cohen, et al., 2008; De Giorgi, et al., 2009), there are a lack of published prospective clinical studies demonstrating that measurement of CTCs alters management such that clinical outcomes are improved. Such clinical outcome studies are currently ongoing. Current guidelines from the National Comprehensive Cancer Network (NCCN) make no recommendations for use of circulating tumor cells.
Guidelines from the American Society for Clinical Oncology (2016) state: "The clinician should not use circulating tumor cells to guide decisions on adjuvant systemic therapy." This is a strong recommendation based upon intermediate-quality evidence.

Scher et al (2015) noted that clinical trials in castration-resistant prostate cancer (CRPC) need new clinical end-points that are valid surrogates for survival. These researchers evaluated circulating tumor cell (CTC) enumeration as a surrogate outcome measure. Examining CTCs alone and in combination with other biomarkers as a surrogate for OS was a secondary objective of COU-AA-301, a multi-national, randomized, double-blind phase III trial of abiraterone acetate plus prednisone versus prednisone alone in patients with metastatic CRPC previously treated with docetaxel. The biomarkers were measured at baseline and 4, 8, and 12 weeks, with 12 weeks being the primary measure of interest. The Prentice criteria were applied to test candidate biomarkers as surrogates for OS at the individual-patient level. A biomarker panel using CTC count and lactate dehydrogenase (LDH) level was shown to satisfy the 4 Prentice criteria for individual-level surrogacy; 12-week surrogate biomarker data were available for 711 patients. The abiraterone acetate plus prednisone and prednisone-alone groups demonstrated a significant survival difference (p = 0.034); surrogate distribution at 12 weeks differed by treatment (p < 0.001); the discriminatory power of the surrogate to predict mortality was high (weighted c-index, 0.81); and adding the surrogate to the model eliminated the treatment effect on survival. Overall, 2-year survival of patients with CTCs less than 5 (low risk) versus patients with CTCs greater than or equal to 5 cells/7.5 ml of blood and LDH greater than 250 U/L (high risk) at 12 weeks was 46 % and 2 %, respectively. The authors concluded that a biomarker panel containing CTC number and LDH level was shown to be a surrogate for survival at the individual-patient level in this trial of abiraterone acetate plus prednisone versus prednisone alone for patients with metastatic CRPC. They stated that independent phase III clinical trials are needed to validate these findings.

An assessment from the Institut National d’Excellence en Santé et Services Sociaux (INESSS) (Arsenault & Le Blanc, 2016) concluded:
"Based on the scientific literature identified, the use of CellSearch tests as a predictive and prognostic biomarker in patients with early-stage breast cancer is not justified. The evidence is insufficient for establishing a concrete association between the presence of CTCs pre- and posttreatment and patient survival. In the case of patients with metastatic breast cancer, the examination of the scientific literature suggests that CTC enumeration prior to treatment could be a prognostic biomarker for patient survival. Despite the prognostic value of CTC enumeration, based on studies, its clinical utility has yet to be confirmed. For now, CellSearch tests should not be used outside the context of a clinical study. Further studies are needed to determine if the CellSearch test could play a clinically significant role in managing breast cancer patients."

**Her-2/neu**

Estrogen and progesterin receptors are important prognostic markers in breast cancer, and the higher the percentage of overall cells positive as well as the greater the intensity, the better the prognosis. Estrogen and progesterone receptor positivity in breast cancer cells is an indication the patient may be a good candidate for hormone therapy. HER-2/neu is an oncogene. Its gene product, a protein, is over-expressed in approximately 20 to 30% of breast cancers. The over-expressed protein is present in unusually high concentration on the surface of some malignant breast cancer cells, causing these cells to rapidly proliferate. It is important because these tumors are susceptible to treatment with Herceptin (trastuzumab), which specifically binds to this over-expressed protein. Herceptin blocks these protein receptors, inhibiting continued replication and tumor growth. HER2/neu may also be expressed in ovarian, gastric, colorectal, endometrial, lung, bladder, prostate, and salivary gland (Chen, et al., 2006).

HER-2/neu is an oncogene encoding a growth factor receptor related to epidermal growth factor receptor (EGFR) and is amplified in approximately 25-30 percent of node-positive breast cancers (Chin, et al. 2006). Overexpression of HER-2/neu is associated with decreased disease-free and overall survival. Overexpression of HER-2/neu may be used to identify patients who
may be may benefit from trastuzumab (Herceptin™) and/or high dose chemotherapy. Trastuzumab is a humanized monoclonal antibody targeting the HER 2/neu (c-erbB-2) oncoprotein.

Her-2 has been used to: assess prognosis of stage II, node positive breast cancer patients; predict disease-free and overall survival in patients with stage II, node positive breast cancer treated with adjuvant cyclophosphamide, doxorubicin, 5-fluorouracil chemotherapy; and determine patient eligibility for Herceptin treatment (Chen, et al., 2006). The College of American Pathologists (CAP) recommends FISH as an optimal method for HER2/neu testing; therefore, positive IHC results are usually confirmed by FISH testing.

There are additional tests that may be used in breast cancer cases, such as DNA ploidy, Ki-67 or other proliferation markers. However, most authorities believe that HER-2/neu, estrogen and progesterone receptor status are the most important to evaluate first. The other tests do not have therapeutic implications and, when compared with grade and stage of the disease, are not independently significant with respect to prognosis.

Harris et al (2007) updated ASCO's recommendations for the use of tumor marker tests in the prevention, screening, treatment, and surveillance of breast cancer. Thirteen categories of breast tumor markers were considered, 6 of which were new for the guideline. The following categories showed evidence of clinical utility and were recommended for use in practice: CA 15-3, CA 27.29, CEA, estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2, urokinase plasminogen activator, plasminogen activator inhibitor 1, and certain multi-parameter gene expression assays. Not all applications for these markers were supported, however. The following categories demonstrated insufficient evidence to support routine use in clinical practice: DNA/ploidy by flow cytometry, p53, cathepsin D, cyclin E (fragments or whole length), proteomics, certain multi-parameter assays, detection of bone marrow micrometastases, and circulating tumor cells (e.g., CellSearch assay). These guidelines found present data insufficient to recommend measurement of Ki67, cyclin D, cyclin E, p27, p21, thymidine
kinase, topoisomerase II, or other markers of proliferation to assign patients to prognostic groups. The guidelines also found insufficient data to recommend assessment of bone marrow micrometastases for management of patients with breast cancer.

Guidelines from the American Society for Clinical Oncology (2016) recommend against the use of soluble HER2 levels to guide selection of type of adjuvant therapy in breast cancer. This is a moderate-strength recommendation based upon low-quality evidence. The guidelines also recommend against the use of HER2 gene coamplification to guide adjuvant chemotherapy selection in breast cancer.

**PancraGen (formerly PathFinderTG - Pancreas)**

PathFinderTG (RedPath Integrated Pathology, Pittsburgh, PA), also known as topographic genotyping, is described by the manufacturer as a quantitative genetic mutational analysis platform for resolving “indeterminate, atypical, suspicious, equivocal and non-diagnostic specimen” diagnoses from pathology specimens (RedPath, 2007). The manufacturer states that PathFinderTG “focuses on acquired mutational damage rather than inherited genetic predisposition for certain diseases, although there are certain NIH recommended inherited conditions for which we do test.” The manufacturer states that the temporal sequence of acquired mutational damage revealed by the PathFinderTG test is an earlier demonstration of tumor biological aggressiveness than current staging systems that rely on the depth of invasion already achieved by the tumor. Most available published evidence for topographic genotyping focuses on retrospective analyses of pathology specimens examining correlations of test results with tumor characteristics (e.g., Saad et al, 2008; Lin et al, 2008; Finkelstein et al, 2003; Pollack et al, 2001; Riberio et al, 1998; Kounelis et al, 1998; Finkelstein et al, 1998; Holst et al, 1998; Jones et al, 1997; Holst et al, 1997; Pricolo et al, 1997; Przygodzki et al, 1997; Finkelstein et al, 1996; Kanbour-shakir et al, 1996; Ribeiro et al, 1996; Pryzgodzki et al, 1996; Safatle-Ribeiro et al, 1996; Papadaki et al, 1996; Przygodzki et al, 1996; Pricolo et al, 1996; Finkelstein et al, 1994). There are no prospective clinical outcome studies on the use of topographic
genotyping in guiding patient management. Current evidence-based guidelines from leading medical professional organizations and public health agencies do not include recommendations for topographic genotyping. In a review on molecular analysis of pancreatic cyst fluid, Shen and colleagues (2009) stated that a large study with validation of PathFinderTG molecular testing of pancreatic fluid will be needed before a firm conclusion can be drawn.

A systematic evidence review of the PathFinderTG prepared for the Agency for Healthcare Research and Quality (Trikalinos, et al., 2010) reviewed evidence available at that time, and found that most studies on loss-of-heterozygosity based topographic genotyping with PathFinderTG were excluded because they only described the molecular profile of different tumors, without assessing the ability of the method to help make diagnosis, prognosis or treatment guidance. The review found no studies that directly measured whether using loss-of-heterozygosity based topographic genotyping with PathFinderTG improves patient-relevant clinical outcomes. The review reported that eligible studies on the diagnostic and prognostic ability of loss-of-heterozygosity based topographic genotyping with PathFinderTG were small in sample sizes and had overt methodological limitations. The review reported that important characteristics of their designs were not clearly reported. The report noted that loss-of-heterozygosity based topographic genotyping with PathFinderTG is claimed to be particularly useful in cases where conventional pathology is unable to provide a conclusive diagnosis. However, the included studies were not designed to address this question. Therefore, it is unclear if the findings of the reviewed studies are directly applicable to patients with the same cancers but with inconclusive diagnosis.

A subsequent study by Panarelli, et al. (2012) comparing PathFinderTG to cytological examination, finding concordance in 35 percent of cases. The authors concluded that the PathFinderTG panel may aid the classification of pancreatic lesions, but is often inaccurate and should not replace cytologic evaluation of these lesions.
The manufacturer has announced that the PathginderTG - Pancreas has been rebranded Pancragen.

Al Haddad, et al. (2015) reported on a multicenter retrospective chart review study to determine the diagnostic accuracy of integrated molecular pathology (Pancragen) for pancreatic adenocarcinoma, and the utility of IMP testing under current guideline recommendations for managing pancreatic cysts. The authors found that Pancragen more accurately determined the malignant potential of pancreatic cysts than a Sendai 2012 guideline management criteria model. Patients who had undergone previous Pancragen testing as prescribed by their physician and for whom clinical outcomes were available from retrospective record review were included (n=492). Performance was determined by correlation between clinical outcome and previous Pancragen diagnosis ("benign"/"statistically indolent" vs. "statistically higher risk [SHR]"/"aggressive") or an International Consensus Guideline (Sendai 2012) criteria model for "surveillance" vs. "surgery." The Cox proportional hazards model determined hazard ratios for malignancy. Benign and statistically indolent Pancragen diagnoses had a 97% probability of benign follow-up for up to 7 years and 8 months from initial Pancragen testing. SHR and aggressive diagnoses had relative hazard ratios for malignancy of 30.8 and 76.3, respectively (both P < 0.0001). Sendai surveillance criteria had a 97% probability of benign follow-up for up to 7 years and 8 months, but for surgical criteria the hazard ratio was only 9.0 (P < 0.0001). In patients who met Sendai surgical criteria, benign and statistically indolent Pancragen diagnoses had a >93% probability of benign follow-up, with relative hazard ratios for SHR and aggressive IMP diagnoses of 16.1 and 50.2, respectively (both P < 0.0001). The authors concluded that Pancragen may improve patient management by justifying more relaxed observation in patients meeting Sendai surveillance criteria.

Loren, et al. (2016) used registry data to determine if initial adjunctive Pancragen testing influenced future real-world pancreatic cyst management decisions for intervention or surveillance relative to 2012 International Consensus Guideline (ICG) recommendations, and if this benefitted patient outcomes.
Using data from the National Pancreatic Cyst Registry, the investigators evaluated associations between real-world decisions (intervention vs. surveillance), ICG model recommendations (surgery vs. surveillance) and Pancragen diagnoses (high-risk vs. low-risk) using 2 x 2 tables. The investigators used Kaplan Meier and hazard ratio analyses to assess time to malignancy. Odds ratios (OR) for surgery decision were determined using logistic regression. Of 491 patients, 206 received clinical intervention at follow-up (183 surgery, 4 chemotherapy, 19 presumed by malignant cytology). Overall, 13 % (66/491) of patients had a malignant outcome and 87 % (425/491) had a benign outcome at 2.9 years' follow-up. When ICG and Pancragen were concordant for surveillance/surgery recommendations, 83 % and 88 % actually underwent surveillance or surgery, respectively. However, when discordant, Pancragen diagnoses were predictive of real-world decisions, with 88 % of patients having an intervention when ICG recommended surveillance but Pancragen indicated high risk, and 55 % undergoing surveillance when ICG recommended surgery but Pancragen indicated low risk. These Pancragen-associated management decisions benefitted patient outcomes in these subgroups, as 57 % had malignant and 99 % had benign outcomes at a median 2.9 years' follow-up. Pancragen was also more predictive of real-world decisions than ICG by multivariate analysis: OR 11.4 (95 % CI 6.0 - 23.7) versus 3.7 (2.4 - 5.8), respectively.

Kowalski, et al. (2016) examined the utility of integrated molecular pathology (IMP) in managing surveillance of pancreatic cysts based on outcomes and analysis of false negatives (FNs) from a previously published cohort (n=492). In endoscopic ultrasound with fine-needle aspiration (EUS-FNA) of cyst fluid lacking malignant cytology, IMP demonstrated better risk stratification for malignancy at approximately 3 years' follow-up than International Consensus Guideline (Fukuoka) 2012 management recommendations in such cases. The investigators reviewed patient outcomes and clinical features of Fukuoka and IMP FN cases. Practical guidance for appropriate surveillance intervals and surgery decisions using IMP were derived from follow-up data, considering EUS-FNA sampling limitations and high-risk clinical circumstances observed. Surveillance intervals...
for patients based on IMP predictive value were compared with those of Fukuoka. Outcomes at follow-up for IMP low-risk diagnoses supported surveillance every 2 to 3 years, independent of cyst size, when EUS-FNA sampling limitations or high-risk clinical circumstances were absent. In 10 of 11 patients with FN IMP diagnoses (2% of cohort), EUS-FNA sampling limitations existed; Fukuoka identified high risk in 9 of 11 cases. In 4 of 6 FN cases by Fukuoka (1% of cohort), IMP identified high risk. Overall, 55% of cases had possible sampling limitations and 37% had high-risk clinical circumstances. Outcomes support more cautious management in such cases when using IMP.

An American Gastroenterological Association Technical Review (Scheiman, et al., 2014) stated: "Testing for molecular alterations in pancreatic cyst fluid is currently available and reimbursed by Medicare under certain circumstances. Case series have confirmed malignant cysts have greater number and quality of molecular alterations, but no study has been properly designed to identify how the test performs in predicting outcome with regard need to surgery, surveillance or predicts interventions leading to improved survival. This adjunct to fine-needle aspiration (FNA) may provide value in distinct clinical circumstances, such as confirmation of a serous lesion due to a lack of KRAS or GNAS mutation in a macrocystic serous cystadenoma, but its routine use is not supported at the present time."

A guideline from the American Society for Gastrointestinal Endoscopy (Muthusamy, et al., 2016) stated: "A more recent study demonstrated that integrated molecular analysis of cyst fluid (ie, combining molecular analysis with results of imaging and clinical features) was able to better characterize the malignant potential of pancreatic cysts compared to consensus guidelines for the management of mucinous cysts [citing Al Haddad, et al., 2015]. ... Molecular analysis (which requires only 200 mL of fluid) may be most useful in small cysts with nondiagnostic cytology, equivocal cyst fluid CEA results, or when insufficient fluid is present for CEA testing [citing Al Haddad, et al., 2014]. However, additional research is needed to determine the precise role molecular analysis of cyst fluid will play in evaluating pancreatic cystic lesions."
Guidelines published in April 2015 by the American Gastroenterological Association (Vege, et al., 2015) have no recommendations for use of topographic genotyping for evaluating pancreatic cysts. Other guidelines (NCCN, 2015; Vege, et al., 2015; Del Chiaro, et al., 2013; Sahani, et al., 2013; Tanaka, et al., 2012) have no firm recommendations for topographic genotyping for assessing indeterminate pancreatic cysts.

The International consensus guidelines for “The management intraductal papillary mucinous neoplasm (IPMN) and mucinous cystic neoplasm (MCN) of the pancreas” (Tanaka et al, 2012) stated that endoscopic ultrasound (EUS) is recommended for all cysts with worrisome features or for cysts greater than 3 cm without these features. Endoscopic US confirmation of a mural nodule, any features of main duct involvement (intraductal mucin or thickened main duct wall), or suspicious or positive cytology for malignancy is an indication for surgical resection. Cysts with high-risk stigmata should be resected in patients medically fit for surgery, although EUS is optional. Endoscopic US can be considered in smaller cysts without worrisome features but is not required. Endoscopic US analysis should include at least cytology, amylase level, and CEA. The guidelines stated that elevated CEA is a marker that distinguishes mucinous from non-mucinous cysts, but not benign from malignant cysts.

*Gene Expression Profiling for Cancer of Unknown Primary*

Carcinoma of unknown primary (CUP) is a biopsy-proven metastatic solid tumor with no primary tumor identified and represents approximately 2% to 4% of all cancer cases. The diagnosis of CUP is made following inconclusive results from standard tests (e.g., biopsy, immunochemistry and other blood work, chest x-rays, and occult blood stool test). The absence of a known primary tumor presents challenges to the selection of appropriate treatment strategies. As a result, patients have a poor prognosis, and fewer than 25% survive 1 year from the time of diagnosis. A variety of tissue-biopsy testing techniques currently are used to determine the origin of the CUP, including immunochemistry; histological examination of specimens stained with eosin and hematoxylin, and electron microscopy. These
techniques definitively identify the type of carcinoma in less than 20% to 30% of CUP.

Gene expression profiling is a technique used to identify the genetic makeup of a tissue sample by characterizing the patterns of mRNA transcribed, or "expressed", by its DNA. Specific patterns of gene expression, reflected in unique configurations of mRNA, are associated with different tumor types. By comparing the gene expression profile (GEP) of an unknown tumor to the profiles of known primary cancers ("referent" profiles), it may be possible to determine the type of tumor from which the CUP originated.

In July 2008, the FDA cleared for marketing the Pathwork Tissue of Origin test (Pathwork Diagnostics, Sunnyvale, CA), a gene expression profiling test that uses microarray processing to determine the type of cancer cells present in a tumor of unknown origin. The test uses the PathChip (Affymetrix Inc., Santa Clara, CA), a custom-designed gene expression array, to measure the expression from 1,668 probe sets to quantify the similarity of tumor specimens to 15 common malignant tumor types, including: bladder, breast, colorectal, gastric, germ cell, hepatocellular, kidney, non-small cell lung, non-Hodgkin's lymphoma, melanoma, ovarian, pancreatic, prostate, soft tissue sarcoma, and thyroid. The degree of correspondence between the tissue sample's GEP and a referent profile is quantified and expressed as a probability-based score.

A multi-center, clinical validation study reported on comparisons of diagnoses based on GEP from 477 banked tissue samples of undifferentiated and poorly differentiated metastases versus standard of care pathology based diagnoses. Comparison of the GEP based diagnoses versus pathology based diagnoses yielded an 89% agreement and the concurrence was greater than 92% for 8 out of 15 types of primary tumors. The overall accuracy of the test was approximately 95% and 98% for positive and negative determinations, respectively (Monzon et al, 2007).

Gene expression profiling is a promising technology in the management of CUP; however, there is insufficient evidence of its
clinical utility compared to that achieved by expert pathologists using current standards of practice. A draft clinical guideline on metastatic malignant disease of unknown origin by the National Institute for Clinical Excellence (NICE, 2010) recommends against using gene expression profiling (e.g., Pathwork TOT, CupPrint, Theros CancerTypeID, miRview Mets) to identify primary tumors in patients with CUP. The guideline explained that currently there is no evidence that gene-expression based profiling improves the management or changes the outcomes for patients with CUP. Guidelines on occult primary from the National Comprehensive Cancer Network (NCCN, 2010) state that, while gene expression profiling looks promising, "prospective clinical trials are necessary to confirm whether this approach can be used in choosing treatment options which would improve the prognosis of patients with occult primary cancers."

An assessment by the Andalusian Agency for Health Technology Assessment (AETSA, 2012) of microRNAs as a diagnostic tool for lung cancer found only two studies assessing the analytical validity of miRview in patients with non-small cell lung cancer. The sensitivity of the miRNA for the detection of carcinoma was between 96% and 100% and the specificity was between 90% and 100%. The area under the ROC curve was close to unity and the positive and negative probability ratios showed a high diagnostic accuracy (9.6 and 0.04, respectively). The assessment stated that, although the quality of the studies was moderate to high, the sensitivity of the diagnostic test may be overestimated as it is a case-control design.

A technology assessment prepared for the Agency for Healthcare Research and Quality (Meleth et al, 2013) found that the clinical accuracy of the PathworkDx, miRview, and CancerTypeID are similar, ranging from 85 percent to 88 percent, and that the evidence that the tests contribute to identifying a tumor of unknown origin was moderate. The assessment concluded that we do not have sufficient evidence to assess the effect of the tests on treatment decisions and outcomes. The assessment noted that most studies of these tests were funded wholly or partially by the manufacturers of these tests, and that the most urgent need in the literature is to have the clinical utility of the
tests evaluated by research groups that have no evidence conflict of interest.

Monzon et al (2009) stated that malignancies found in unexpected locations or with poorly differentiated morphologies can pose a significant challenge for tissue of origin determination. Current histologic and imaging techniques fail to yield definitive identification of the tissue of origin in a significant number of cases. The aim of this study was to validate a predefined 1,550-gene expression profile for this purpose. Four institutions processed 547 frozen specimens representing 15 tissues of origin using oligonucleotide microarrays were used in this study. Half of the specimens were metastatic tumors, with the remainder being poorly differentiated and undifferentiated primary cancers chosen to resemble those that present as a clinical challenge. In this blinded multi-center validation study the 1,550-gene expression profile was highly informative in tissue determination. The study found overall sensitivity (positive percent agreement with reference diagnosis) of 87.8 % (95 % CI: 84.7 % to 90.4 %) and overall specificity (negative percent agreement with reference diagnosis) of 99.4 % (95 % CI: 98.3 % to 99.9 %). Performance within the subgroup of metastatic tumors (n = 258) was found to be slightly lower than that of the poorly differentiated and undifferentiated primary tumor subgroup, 84.5 % and 90.7 %, respectively (p = 0.04). Differences between individual laboratories were not statistically significant. The authors concluded that this study represents the first adequately sized, multi-center validation of a gene-expression profile for tissue of origin determination restricted to poorly differentiated and undifferentiated primary cancers and metastatic tumors. These results indicate that this profile should be a valuable addition or alternative to currently available diagnostic methods for the evaluation of uncertain primary cancers.

Monzon and Koen (2010) stated that tumors of uncertain or unknown origin are estimated to constitute 3 % to 5 % of all metastatic cancer cases. Patients with these types of tumors show worse outcomes when compared to patients in which a primary tumor is identified. New molecular tests that identify molecular signatures of a tissue of origin have become available.
The authors reviewed the literature on existing molecular approaches to the diagnosis of metastatic tumors of uncertain origin and discuss the current status and future developments in this area. Published peer-reviewed literature, available information from medical organizations (NCCN), and other publicly available information from tissue-of-origin test providers and/or manufacturers were used in this review. The authors concluded that molecular tests for tissue-of-origin determination in metastatic tumors are available and have the potential to significantly impact patient management. However, available validation data indicate that not all tests have shown adequate performance characteristics for clinical use. Pathologists and oncologists should carefully evaluate claims for accuracy and clinical utility for tissue-of-origin tests before using test results in patient management. The personalized medicine revolution includes the use of molecular tools for identification/confirmation of the site of origin for metastatic tumors, and in the future, this strategy might also be used to determine specific therapeutic approaches.

Anderson and Weiss (2010) noted that pathologists use various panels of IHC stains to identify the site of tissue of origin for metastatic tumors, particularly poorly or undifferentiated cancers of unknown or uncertain origin. Although clinicians believe that immunostains contribute greatly to determining the probable primary site among 3 or more possibilities, objective evidence has not been convincingly presented. This meta-analysis reviews the objective evidence supporting this practice and summarizes the performance reported in 5 studies published between 1993 and 2007. A literature search was conducted to identify IHC performance studies published since 1990 that were masked, included more than 3 tissues types, and used more than 50 specimens. The 5 studies found in this search were separated into 2 subgroups for analysis: those, which included only metastatic tumors (n = 368 specimens) and the blended studies, which combined primary tumors and metastases (n = 289 specimens). The meta-analysis found that IHCs provided the correct tissue identification for 82.3 % (95 % CI: 77.4 % to 86.3 %) of the blended primary and metastatic samples and 65.6 % (95 % CI: 60.1 % to 70.7 %) of metastatic cancers. This difference is
both clinically and statistically significant. The authors concluded that this literature review confirms that there is still an unmet medical need in identification of the primary site of metastatic tumors. It establishes minimum performance requirements for any new diagnostic test intended to aid the pathologist and oncologist in tissue of origin determination.

**GeneSearch BLN**

The presence of breast tumor cells in axillary lymph nodes is a key prognostic indicator in breast cancer. During surgery to remove breast tumors, patients often undergo biopsy of the sentinel (i.e., first) node(s) that receive lymphatic fluid from the breast. Excised sentinel lymph nodes are currently evaluated post-operatively by formalin-fixed paraffin-embedded Hematoxylin and Eosin (H&E) histology and IHC. GeneSearch™ Breast Lymph Node (BLN) assay (Veridex, LLC, Warren, NJ) is a novel method to examine the extracted sentinel lymph nodes for metastases and can provide information during surgery within 30 to 40 minutes from the time the sentinel node is removed, potentially avoiding a second operation for some patients. The GeneSearch BLN assay received FDA pre-market approval on July 16, 2007 as a qualitative in vitro diagnostic test for the rapid detection of metastases larger than 0.2 mm in nodal tissue removed from sentinel lymph node biopsies of breast cancer patients. GeneSearch BLN assay uses real time reverse transcriptase polymerase chain reaction (RT-PCR) to detect the gene expression markers, mammaglobin (MG) and cytokeratin 10 (CI19), which are abundant in breast tissue but scarce in lymph node cells. In the clinical trial conducted by Veridex, which was submitted to the FDA, the sensitivity of the GeneSearch BLN Assay was reported to be 87.6 % and the specificity was 94.2 % (Julian et al, 2008). According to the product labeling, "The GeneSearch™ Breast Lymph Node (BLN) assay may be used in conjunction with sentinel lymph node biopsy for a patient who has been counseled on use of this test and has been informed of its performance. False positive results may be associated with increased morbidity. False negative and inconclusive test results may be associated with delayed axillary node dissection. Clinical studies so far are inconclusive about a benefit from treatment based on findings of breast cancer micro-
metastases in sentinel lymph nodes."

Blumencranz et al (2007) compared the GeneSearch BLN assay with results from conventional histologic evaluation from 416 patients at 11 clinical sites and reported that the GeneSearch BLN assay detected 98 % of metastases greater than 2 mm in size and 57 % of metastasis less than 0.2 mm. False positives were reported in 4 % of the cases. However, there were several limitations of this study, including the lack of a description of patient recruitment, inadequate descriptions of several analyses performed, substantial variations in test performance across sites, and ad hoc comparison of the assay to other intra-operative techniques.

Viale et al (2008) analyzed 293 lymph nodes from 293 patients utilizing the GeneSearch BLN assay. Using histopathology as the reference standard, the authors reported that the BLN assay correctly identified 51 of 52 macro-metastatic and 5 of 20 micro-metastatic sentinel lymph nodes (SLNs), with a sensitivity of 98.1 % to detect metastases larger than 2 mm, 94.7 % for metastases larger than 1 mm, and 77.8 % for metastases larger than 0.2 mm. The overall concordance with histopathology was 90.8 %, with a specificity of 95.0 %, a positive predictive value of 83.6 %, and a negative predictive value of 92.9 %. When the results were evaluated according to the occurrence of additional metastases to non-SLN in patients with histologically positive SLNs, the assay was positive in 33 (91.7 %) of the 36 patients with additional metastases and in 22 (66.6 %) of the 33 patients without further echelon involvement. The authors concluded that the sensitivity of the GeneSearch BLN assay is comparable to that of the histopathologic examination of the entire SLN by serial sectioning at 1.5 to 2 mm.

Although treatment for metastases larger than 2.0 mm is widely accepted as beneficial, clinical studies have not yet provided data for a consensus on benefit from treatment based on very small breast cancer metastases (between 0.2 mm and 2.0 mm) in SLNs. False positive results may be associated with increased morbidity, usually due to effects of axillary node dissection surgery. Patients who undergo axillary lymph node dissection (ALND) have
significantly higher rates of increased swelling in the upper arm and forearm (lymphedema), pain, numbness, and motion restriction about the shoulder when compared with patients who undergo only sentinel lymph node dissection (SLND). False negative and inconclusive test results may be associated with delayed axillary node dissection. Clinical studies so far are inconclusive regarding a benefit from treatment based on findings of breast cancer micro-metastases in SLNs. Preliminary data suggest that the GeneSearch BLN assay has high specificity and moderate sensitivity when only macro-metastases are included in the analysis. The clinical significance of micro-metastases is still being debated in the literature, thus, the failure of the GeneSearch BLN assay to perform adequately in the detection of micro-metastases is of unknown significance.

A systematic evidence review by the BlueCross BlueShield Association Technology Evaluation Center (BCBSA, 2007) determined that the use of the GeneSearch BLN assay to detect sentinel node metastases in early stage breast cancer does not meet the TEC criteria. The assessment stated, "There are several operational issues that add difficulty to the use of the GeneSearch assay, including the need for fresh specimens (rather than putting them in formalin for permanent fixation), the learning curve involved in reducing both the percentage of invalid results (from about 15% initially to 4 - 8% for more experienced technicians) and the time to perform the test compared to alternative intra-operative techniques (which take less than 15 minutes)." Furthermore, the assessment stated "The GeneSearch assay also provides less information for staging than other intra-operative procedures, since it cannot distinguish between micro- and macro-metases. Nor can it indicate the location of the metastasis (inside or outside the node). Post-operative histology is therefore required in all cases. It is less crucial when frozen section histology is performed, since pathologists can judge the size of the metastasis and its location from this test, although distortion is possible. To summarize, the data available is inadequate to assess the clinical utility of the GeneSearch assay compared to either post-operative histology alone or to the alternative intra-operative tests such as imprint cytology and frozen section histology. In addition, the balance of benefits
versus harms may require higher specificity to avoid unnecessary ALNDs and their sequelae, whereas the GeneSearch design emphasizes sensitivity."

A report by Adelaide Health Technology Assessment stated that, if the GeneSearch BLN Assay is to play a role in reducing the mortality of breast cancer patients, it will be through more accurate diagnosis of breast cancer metastasis during SNB (Ellery, et al., 2010). The report noted, however, that, as yet there are no data to indicate whether SNB itself lowers the mortality rate among breast cancer patients. Hence, it is unclear whether the GeneSearch BLN Assay would have any indirect effect on breast cancer mortality until further investigation into SNB concludes.

Thus, there is insufficient evidence to make a conclusion about the effectiveness of the GeneSearch BLN assay. The FDA is requiring the manufacturer to conduct two post-approval studies. The primary objective of the first study is to estimate the positive predictive concordance between the GeneSearch BLN assay and histology as routinely practiced and the objectives of the second clinical study are (i) determine the assay turn-around-time from the time of node removal to the report of the assay result to the surgeon and (ii) determine whether the assay result was or was not received in time to make an intra-operative decision and (iii) collect data in relation to other surgical procedures during the sentinel lymph node dissection/breast surgery to determine if the assay turn-around-time resulted in longer surgery time.

**BT Test**

Provista Life Sciences (Phoenix, AZ) has developed a laboratory test called the Biomarker Translation Test, or the BT Test, which is a test score based on a multi-protein biomarker analysis (i.e., IL-2, -6,-8,-12, TNFa, EGF, FGF, HGF, VEGF) and medical profile of an individual's risk factors for breast cancer. It is intended to be used as an adjunctive test along with other breast cancer detection modalities, however, there are no published studies of the effectiveness of this test.
Bcl-2

Bcl-2 (B-cell CLL/lymphoma 2; BCL2) is a proto-oncogene whose protein product, bcl-2, suppresses programmed cell death (apoptosis), resulting in prolonged cellular survival without increasing cellular proliferation. Dysregulation of programmed cell death mechanisms plays a role in the pathogenesis and progression of cancer as well as in the responses of tumors to therapeutic interventions. Many members of the Bcl-2 family of apoptosis-related genes have been found to be differentially expressed in various malignancies (Reed, 1997).

Salgia (2008) reviewed the evidence for detection of Bcl-2 in lung cancer. The author observed that Bcl-2 over-expression has been reported in 22 to 56% of lung cancers with a higher expression in squamous cell carcinoma as compared to adenocarcinoma histology. The author concluded, however, that the association of Bcl-2 expression and prognosis in non-small cell lung cancer is unclear. Multiple reports have demonstrated that Bcl-2-positive lung cancers are associated with a superior prognosis compared to those that are Bcl-2 negative. However, other studies have failed to demonstrate any survival impact with bcl-2 positivity, while over-expression has also been associated with a poorer outcome. A meta-analysis that included 28 studies examining the prognostic influence of Bcl-2 in non-small cell lung cancer concluded that over-expression of Bcl-2 was associated with a significantly better prognosis in surgically resected (hazard ratio 0.5, 95% CI 0.39-0.65).

Compton (2008) recently reviewed the evidence on the Bcl-2 oncogene and other tumor markers in colon cancer. Compton explained that Bcl-2 is a gene related to apoptosis/cell suicide. Bcl-2 over-expression leading to inhibition of cell death signaling has been observed as a relatively early event in colorectal cancer development. The author concluded that the independent influence of the Bcl-2 oncogene on prognosis remains unproven, and explained that the variability in assay methodology, conflicting results from various studies examining the same factor, and the prevalence of multiple small studies that lack statistically robust, multivariate analyses all contribute to the lack of
conclusive data. Compton concluded that before the Bcl-2 oncogene and certain other tumor markers can be incorporated into clinically meaningful prognostic stratification systems, "more studies are required using multivariate analysis, well-characterized patient populations, reproducible and current methodology, and standardized reagents."

**CD31**

Compton (2008) reviewed the evidence for intratumor microvessel density (MVD) and antibodies against CD31 in colorectal cancer. The author explained that intratumoral MVD is a reflection of tumor-induced angiogenesis. Microvessel density has been independently associated with shorter survival in some, but not all studies. A meta-analysis of all studies relating MVD expression to prognosis concluded that at least some of the variability could be explained by the different methods of MVD assessment. The author noted that there was a significant inverse correlation between immunohistochemical expression and survival when MVD was assessed using antibodies against CD31 or CD34, but not factor VIII. The author concluded, however, that there is a need for evaluation of MVD in large studies of prognostic factors using multivariate analysis; however, standard guidelines for staining, evaluation, and interpretation of MVD are lacking.

In a review, Hayes (2008) reviewed the evidence for assessing angiogenesis factors in breast cancer. The author noted that, in an early report, MVD count (as indicated by IHC staining for endothelial cells, such as factor VIII-related antigen or CD31) was a statistically significant independent predictor of both disease-free and overall survival in women with both node-negative and node-positive breast cancer. The author noted, however, that subsequent data are conflicting, with some studies confirming and others refuting the initial findings. The author stated that, "As with many of the other tumor marker studies, evaluation of angiogenesis is complicated by technical variation, reader inconsistency, and potential interaction with therapy."

Burgdorf (2006) reviewed the use of CD31 in acquired progressive
lymphangioma. The author stated that special staining techniques reveal that the cells are variably positive for CD31, but that the staining patterns are too variable to be of diagnostic importance.

Some authorities have stated that CD31 staining may be useful for diagnosing angiosarcomas (Schwartz, 2008; Carsi and Sim, 2008; Fernandez and Schwartz, 2007; McMains and Gourin, 2007). CD31 immunostaining can help confirm that the tumor originates from blood vessels.

**TOP2A**

Topoisomerase II alpha is a protein encoded by the TOP2A gene and is proposed as a predictive and prognostic marker for breast cancer. It is also proposed as an aid in predicting response to anthracycline therapy in breast cancer. Two types of tests are available for topoisomerase II alpha: topoisomerase II alpha protein expression testing by immunohistochemistry (IHC); and TOP2A gene amplification testing by FISH (eg, TOP2A FISH pharmDx Assay).

The topoisomerase II alpha gene (TOP2A) is located adjacent to the HER-2 oncogene at the chromosome location 17q12-q21 and is either amplified or deleted (with equal frequency) in a great majority of HER-2 amplified primary breast tumors and also in tumors without HER-2 amplification. Recent experimental as well as numerous, large, multi-center trials suggest that amplification (and/or deletion) of TOP2A may account for both sensitivity or resistance to commonly used cytotoxic drugs (e.g., anthracyclines) depending on the specific genetic defect at the TOP2A locus. An analysis of TOP2A aberrations in the Danish Breast Cancer Cooperative Group trial 89D (Nielsen, et al., 2008) suggested a differential benefit of adjuvant chemotherapy in patients with primary breast cancer, favoring treatment with epirubicin in patients with TOP2A amplifications, and perhaps deletions; however, the authors concluded that, "Additional studies are needed to clarify the exact importance of TOP2A deletions on outcome, but deletions have proven to be associated with a very poor prognosis."
The National Comprehensive Cancer Network (NCCN, 2008) guideline on breast cancer does not address the use of TOP2A testing. Guidelines from the American Society for Clinical Oncology (2016) state: "The clinician should not use TOP2A gene amplification or TOP2A protein expression by IHC to guide adjuvant chemotherapy selection. This is a moderate-strength recommendation based upon high quality evidence. The guidelines also recommend against the use of TOP2A gene coamplification to guide adjuvant chemotherapy selection.

TSP-1

Ghoneim et al (2008) explained that thrombospondin-1 (TSP-1) is a member of a family of five structurally related extracellular glycoproteins that plays a major role in cell-matrix and cell to cell interactions. Due to its multifunctional nature and its ability to bind to a variety of cell surface receptors and matrix proteins, TSP-1 has been identified as a potential regulator of angiogenesis and tumor progression. Data collected by Secord, et al. (2007) suggested that high THBS-1 levels may be an independent predictor of worse progression-free and overall survival in women with advanced-stage epithelial ovarian cancer. However, a phase II clinical trial (Garcia, et al., 2008) of bevacizumab and low-dose metronomic oral cyclophosphamide in recurrent ovarian cancer reported that levels of TSP-1 were not associated with clinical outcome.

mdr1

In a review on multidrug resistance in acute leukemia, List and Spier (1992) explained that the mdr1 gene or its glycoprotein product, P-glycoprotein, is detected with high frequency in secondary acute myeloid leukemia (AML) and poor-risk subsets of acute lymphoblastic leukemia. Investigations of mdr1 regulation in normal hematopoietic elements have shown a pattern that corresponds to its regulation in acute leukemia, explaining the linkage of mdr1 to specific cellular phenotypes. Therapeutic trials are now in progress to test the ability of various MDR-reversal agents to restore chemotherapy sensitivity in high-risk acute leukemias.
In a phase III multi-center randomized study to determine whether quinine would improve the survival of adult patients with de novo AML, Soary et al (2003) reported that neither mdr1 gene or P-glycoprotein expression influenced clinical outcome.

A phase I/II study of the MDR modulator Valspodar (PSC 833, Novartis Pharma) combined with daunorubicin and cytarabine in patients with relapsed and primary refractory acute myeloid leukemia (Gruber et al, 2003) reported that P-glycoprotein did not give an obvious improvement to the treatment results.

**MRP-1**

Motility-related protein (MRP-1) is a glycoprotein with a sequence identical to that of CD9, a white blood cell differentiation antigen. The level of MRP-1/CD9 expression has been found in investigational studies to inhibit cell motility and low MRP-1/CD9 expression may be associated with the metastatic potential of breast cancer (Miyake et al, 1995). CD9 immuno-expression is also being investigated as a potential new predictor of tumor behavior in patients with squamous cell carcinoma of the head and neck (Mhawech et al, 2004) as well as other tumors (e.g., urothelial bladder carcinoma, colon cancer, lung cancer); however, prospective studies are needed to determine the clinical role of MRP-1/CD9 expression in tumors.

**PLAP**

The National Comprehensive Cancer Network's guideline on occult primary tumors includes placental alkaline phosphatase (PLAP) as a useful marker to assist in identifying germ cell seminoma and non-seminoma germ cell tumors in unknown primary cancer (NCCN, 2009).

**MPO**

Myeloperoxidase (MPO), a blood protein, is a major component of azurophilic granules of neutrophils. Myeloperoxidase analysis has been used to distinguish between the immature cells in acute myeloblastic leukemia (cells stain positive) and those in acute
lymphoblastic leukemia (cells stain negative). The National Comprehensive Cancer Network guidelines on acute myeloid leukemia (AML) include MPO analysis in the classification of AML (NCCN, 2008).

Matsuo et al (2003) examined the prognostic factor of the percentage of MPO-positive blast cells for AML. Cytochemical analysis of MPO was performed in 491 patients who were registered to the Japan Adult Leukemia Study Group (AML92 study). Patients were divided into two groups using the percentage of MPO-positive blast (high [ > or = 50%] and low [< 50%]). Complete remission rates were 85.4% in the former and 64.1% in the latter (p = 0.001). The OS and DFS were significantly better in the high MPO group (48.3 versus 18.7% for OS, and 36.3 versus 20.1% for DFS, p < 0.001, respectively). Multi-variate analysis showed that both karyotype and the percentage of MPO-positive blast cells were equally important prognostic factors. The high MPO group still showed a better survival even when restricted to the intermediate chromosomal risk group or the patients with normal karyotype (p < 0.001). The OS of patients with normal karyotype in the high MPO group was almost equal to that of the favorable chromosomal risk group. The authors concluded that the percentage of MPO-positive blast cells is a simple and highly significant prognostic factor for AML patients, and especially useful to stratify patients with normal karyotype.

DCP

The most commonly used marker for hepatocellular carcinoma (HCC) is the AFP level. Des-gamma-carboxy prothrombin (DCP) (also known as "prothrombin produced by vitamin K absence or antagonism II" [PIVKA II]) has also shown promise in the diagnosis of HCC (Toyoda et al, 2006; Ikoma et al, 2002; Nomura et al, 1996; Liebman et al, 1984). In one series of 76 patients with HCC, this marker was elevated in 69 patients with a mean serum concentration of 900 mcg/L. Much lower mean values were seen in patients with chronic active hepatitis, metastatic disease to the liver, and normal subjects (10 and 42 mcg/L and undetectable, respectively) (Liebman et al, 1984). Elevations in serum levels of
DCP are less frequent in tumors less than 3 cm in size (Nakamura et al, 2006; Weitz and Liebman, 1993). Aoyagi et al (1996) as well as Weitz and Liebman (1993) reported that abnormal prothrombin levels do not correlate well with serum AFP.

Toyoda et al (2006) measured AFP, lens culinaris agglutinin A-reactive fraction of AFP (AFP-L3), and DCP for the evaluation of tumor progression and prognosis of patients with HCC (n = 685) at the time of initial diagnosis. Positivity for AFP > 20 ng/dL, AFP-L3 > 10% of total AFP, and/or DCP > 40 mAU/mL was determined. In addition, tumor markers were measured after treatment of HCC. Of the 685 patients, 337 (55.8%) were positive for AFP, 206 (34.1%) were positive for AFP-L3, and 371 (54.2%) were positive for DCP. In a comparison of patients positive for only 1 tumor marker, patients positive for AFP-L3 alone had a greater number of tumors, whereas patients positive for DCP alone had larger tumors and a higher prevalence of portal vein invasion. When patients were compared according to the number of tumor markers present, the number of markers present clearly reflected the extent of HCC and patient outcomes. The number of markers present significantly decreased after treatment. The authors concluded that tumor markers AFP-L3 and DCP appeared to represent different features of tumor progression in patients with HCC and that the number of tumor markers present could be useful for the evaluation of tumor progression, prediction of patient outcome, and treatment efficacy.

The National Comprehensive Cancer Network's guideline on HCC (NCCN, 2008) does not include measurement of DCP among the surveillance test options for HCC. According to NCCN guidelines, proposed surveillance for the early detection of HCC among high-risk populations (e.g., chronic hepatitis C virus-infected patients) includes liver ultrasonography every 3 to 6 months and evaluation of alkaline phosphatase, albumin, and AFP. The guidelines stated, "It is not yet clear if early detection of hepatocellular cancer with routine screening improves the percentage of patients detected with disease at a potentially curative stage, but high-risk chronic hepatitis C virus-infected patients should be considered for ongoing recurrent screening until these issues have been resolved. The level of des-gamma-carboxy-prothrombin protein
induced by vitamin K absence (PIVKA-II) is also increased in many patients with hepatocellular carcinoma. However, as is true with AFP, PIVKA-II may be elevated in patients with chronic hepatitis. Furthermore, according to Sherman (2008), DCP has not been adequately studied as a screening test for HCC and cannot be recommended at this time.

NMP66

Researchers at Matritech (Newton, MA) have detected the presence of nuclear matrix protein (NMP) in the blood of women at the early stage of breast cancer, which is absent in the blood of healthy women, as well as those with fibroadenoma. NMP66 has been selected as a marker for further development and clinical trials of a test for use in the detection and monitoring of women with, or at risk for, breast cancer have been initiated (Wright and McGechan, 2003). However, there are no published studies on the effectiveness of NMP66 testing at this time.

HERmark

HERmark Breast Cancer Assay (biosciences monogram) is used to help determine prognosis and therapeutic choices for metastatic breast cancer (Raman, et al., 2013). Clinical practice guidelines recommend determining HER2 status in patients with all invasive breast cancer, but caution that current HER2 testing methods such as central immunohistochemistry and Fluorescence in situ Hybridization test may be inaccurate in approximately 20% of cases. According to the HERmark Web site, their method precisely quantifies HER2 total protein and HER2 homodimer levels in formalin-fixed, paraffin-embedded tissue sections and outperformed Fluorescence in situ Hybridization at determining patient outcomes in patients with metastatic breast cancer.

HERmark testing has been proposed for a number of indications, including use to predict response to trastuzumab in the treatment of metastatic breast cancer. Monogram, the manufacturer of the HERmark test, claims that the test can provide a more precise and quantitative measurement of the HER2 gene than IHC and fluorescent in-situ hybridization (FISH) tests. The HERmark
provides a quantitative measurement of HER2 total protein and HER2 homodimer levels, while conventional methods are an indirect measure of the HER2 gene, the manufacturer claims. The HERmark test will be offered as a CLIA-validated assay through Monogram's CAP-certified clinical laboratory. Other proposed indications for HERmark include determining the prognosis for breast cancer, and predicting treatment results in cancers other than breast cancer (e.g., ovarian, prostate, head and neck, etc.). There are no current recommendations from leading medical professional organizations for the use of HERmark testing for breast cancer.

Yardley, et al. (2015) compared quantitative HER2 expression by the HERmark Breast Cancer Assay (HERmark) with routine HER2 testing by immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH), and correlated HER2 results with overall survival (OS) of breast cancer patients in a multicenter Collaborative Biomarker Study (CBS). Two hundred and thirty-two formalin-fixed, paraffin-embedded breast cancer tissues and local laboratory HER2 testing results were provided by 11 CBS sites. HERmark assay and central laboratory HER2 IHC retesting were retrospectively performed in a blinded fashion. HER2 results by all testing methods were obtained in 192 cases. HERmark yielded a continuum of total HER2 expression (H2T) ranging from 0.3 to 403 RF/mm² (approximately 3 logs). The distribution of H2T levels correlated significantly (P<0.0001) with all routine HER2 testing results. The concordance of positive and negative values (equivocal cases excluded) between HERmark and routine HER2 testing was 84% for local IHC, 96% for central IHC, 85% for local FISH, and 84% for local HER2 status. OS analysis revealed a significant correlation of shorter OS with HER2 positivity by local IHC (HR=2.6, P=0.016), central IHC (HR=3.2, P=0.015), and HERmark (HR=5.1, P<0.0001) in this cohort of patients most of whom received no HER2-targeted therapy. The OS curve of discordant low (HER2 positive but H2T low, 10% of all cases) was aligned with concordant negative (HER2 negative and H2T low, HR=1.9, P=0.444), but showed a significantly longer OS than concordant positive (HER2 positive and H2T high, HR=0.31, P=0.024). Conversely, the OS curve of discordant high (HER2 negative but H2T high, 9% of all cases) was aligned with
concordant positive (HR=0.41, P=0.105), but showed a significantly shorter OS than concordant negative (HR=41, P<0.0001).

**MDM2**

Noon et al (2010) stated that renal cell carcinoma (RCC) is the most common type of kidney cancer and follows an unpredictable disease course. These researchers reviewed 2 critical genes associated with disease progression -- p53 and murine double minute 2 (MDM2) -- and provided a comprehensive summary and critical analysis of the literature regarding these genes in RCC. Information was compiled by searching the PubMed database for articles that were published or e-published up to April 1, 2009. Search terms included renal cancer, renal cell carcinoma, p53, and MDM2. Full articles and any supplementary data were examined; and, when appropriate, references were checked for additional material. All studies that described assessment of p53 and/or MDM2 in renal cancer were included. The authors concluded that increased p53 expression, but not p53 mutation, is associated with reduced overall survival/more rapid disease progression in RCC. There also was evidence that MDM2 up-regulation is associated with decreased disease-specific survival. Two features of RCC stood out as unusual and will require further investigation: (i) decreased disease-specific survival. Two features of RCC stood out as unusual and will require further investigation: (i)increased p53 expression is tightly linked with increased MDM2 expression; and (ii) patients who have tumors that display increased p53 and MDM2 expression may have the poorest overall survival. Because there was no evidence to support the conclusion that p53 mutation is associated with poorer survival, it seemed clear that increased p53 expression in RCC occurs independent of mutation. The authors stated that further investigation of the mechanisms leading to increased p53/MDM2 expression in RCC may lead to improved prognostication and to the identification of novel therapeutic interventions.

**OVA1**
OVA1 is a blood test used to aid in the evaluation of pelvic masses for the likelihood of malignancy before surgery. OVA1 measures five biomarkers: apolipoprotein A1 (Apo A-1), beta-2 microglobulin (B2M), CA-125, prealbumin, and transferrin. The results of these measurements are applied to an algorithm, resulting in a numerical score.

The OVA1 Test (Vermillion Inc. and Quest Diagnostics) is a serum test that is intended to help physicians determine if a woman is at risk for a malignant pelvic mass prior to biopsy or exploratory surgery, when the physician’s independent clinical and radiological evaluation does not indicate malignancy (Mundy, et al., 2010). The OVA1 Test employs an in vitro diagnostic multivariate index (IVDMIA) that combines the results of five immunoassays to produce a numerical score indicating a woman’s likelihood of malignancy. The OVA1 Test is intended to help physicians assess if a pelvic mass is benign or malignant in order to help determine whether to refer a woman to a gynecologic oncologist for surgery. The OVA1 Test was cleared by the FDA for use in women who meet the following criteria: over age 18, ovarian adnexal mass present for which surgery is planned, and not yet referred to an oncologist. The intended use of the OVA1 Test is an aid to further assess the likelihood that malignancy is present when the physician’s independent clinical and radiological evaluation does not indicate malignancy. According to the product labeling, the OVA1 Test is not intended as a screening or stand-alone diagnostic assay. There is a lack of evidence in the peer-reviewed published medical literature on the OVA1 Test.

Ueland, et al. (2011) sought to compare the effectiveness of physician assessment with the OVA1 multivariate index assay in identifying high-risk ovarian tumors. The multivariate index assay was evaluated in women scheduled for surgery for an ovarian tumor in a prospective, multi-institutional trial involving 27 primary-care and specialty sites throughout the United States. Preoperative serum was collected, and results for the multivariate index assay, physician assessment, and CA 125 were correlated with surgical pathology. Physician assessment was documented by each physician before surgery. CA 125 cutoffs were chosen in
accordance with the referral guidelines of the American College of Obstetricians and Gynecologists. The study enrolled 590 women, with 524 evaluable for the multivariate index assay and CA 125, and 516 for physician assessment. Fifty-three percent were enrolled by nongynecologic oncologists. There were 161 malignancies and 363 benign ovarian tumors. Physician assessment plus the multivariate index assay correctly identified malignancies missed by physician assessment in 70% of nongynecologic oncologists, and 95% of gynecologic oncologists. The multivariate index assay also detected 76% of malignancies missed by CA 125. Physician assessment plus the multivariate index assay identified 86% of malignancies missed by CA 125, including all advanced cancers. The investigators stated that the performance of the multivariate index assay was consistent in early- and late-stage cancers.

Ware Miller, et al. (2011) sought to estimate the performance of the ACOG referral guidelines for pelvic mass with the OVA1 multivariate index assay. A prospective, multi-institutional trial included 27 primary care and specialty sites throughout the United States. The College guidelines were evaluated in women scheduled for surgery for an ovarian mass. Clinical criteria and blood for biomarkers were collected before surgery. A standard CA 125-II assay was used and the value applied to the multivariate index assay algorithm and the CA 125 analysis. Study results were correlated with surgical pathology. Of the 590 women enrolled with ovarian mass on pelvic imaging, 516 were evaluable. There were 161 malignancies (45 premenopausal and 116 postmenopausal). The College referral criteria had a modest sensitivity in detecting malignancy. Replacing CA 125 with the multivariate index assay increased the sensitivity (77-94%) and negative predictive value (87-93%) while decreasing specificity (68-35%) and positive predictive value (52-40%). Similar trends were noted for premenopausal women and early-stage disease.

Bristow, et al. (2013) sought to validate the effectiveness of a multivariate index assay in identifying ovarian malignancy compared to clinical assessment and CA125-II, among women undergoing surgery for an adnexal mass after enrollment by non-gynecologic oncology providers. A prospective, multi-institutional
trial enrolled female patients scheduled to undergo surgery for an adnexal mass from 27 non-gynecologic oncology practices. Preoperative serum samples and physician assessment of ovarian cancer risk were correlated with final surgical pathology. A total of 494 subjects were evaluable for multivariate index assay, CA125-II, and clinical impression. Overall, 92 patients (18.6%) had a pelvic malignancy. Primary ovarian cancer was diagnosed in 65 patients (13.2%), with 43.1% having FIGO stage I disease. For all ovarian malignancies, the sensitivity of the multivariate index assay was 95.7% (95%CI=89.3-98.3) when combined with clinical impression. The multivariate index assay correctly predicted ovarian malignancy in 91.4% (95%CI=77.6-97.0) of cases of early-stage disease, compared to 65.7% (95%CI=49.2-79.2) for CA125-II. The multivariate index assay correctly identified 83.3% malignancies missed by clinical impression and 70.8% cases missed by CA125-II. Multivariate index assay was superior in predicting the absence of an ovarian malignancy, with a negative predictive value of 98.1% (95%CI=95.2-99.2). Both clinical impression and CA125-II were more accurate at identifying benign disease. The multivariate index assay correctly predicted benign pathology in 204 patients (50.7%, 95%CI=45.9-55.6) when combined with clinical impression.

Longoria, et al. (2014) sought to analyze the effectiveness of the OVA1 multivariate index assay (MIA) in identifying early-stage ovarian malignancy compared to clinical assessment, CA 125-II, and modified American Congress of Obstetricians and Gynecologists (ACOG) guidelines among women undergoing surgery for an adnexal mass. Patients were recruited in 2 related prospective, multi-institutional trials involving 44 sites. All women had preoperative imaging and biomarker analysis. Preoperative biomarker values, physician assessment of ovarian cancer risk, and modified ACOG guideline risk stratification were correlated with surgical pathology. A total of 1016 patients were evaluable for MIA, CA 125-II, and clinical assessment. Overall, 86 patients (8.5%) had primary-stage I/II primary ovarian malignancy, with 70.9% having stage I disease and 29.1% having stage II disease. For all early-stage ovarian malignancies, MIA combined with clinical assessment had significantly higher sensitivity (95.3%; 95% confidence interval [CI], 88.6-98.2) compared to clinical
assessment alone (68.6%; 95% CI, 58.2-77.4), CA 125-II (62.8%; 95% CI, 52.2-72.3), and modified ACOG guidelines (76.7%; 95% CI, 66.8-84.4) (P < .0001). Among the 515 premenopausal patients, the sensitivity for early-stage ovarian cancer was 89.3% (95% CI, 72.8-96.3) for MIA combined with clinical assessment, 60.7% (95% CI, 42.4-76.4) for clinical assessment alone, 35.7% (95% CI, 20.7-54.2) for CA 125-II, and 78.6% (95% CI, 60.5-89.8) for modified ACOG guidelines. Early-stage ovarian cancer in postmenopausal patients was correctly detected in 98.3% (95% CI, 90.9-99.7) of cases by MIA combined with clinical assessment, compared to 72.4% (95% CI, 59.8-82.2) for clinical assessment alone, 75.9% (95% CI, 63.5-85.0) for CA 125-II, and 75.9% (95% CI, 63.5-85.0) for modified ACOG guidelines.

Bristow, et al. (2013) assessed the impact on referral patterns of using the OVA1 Multivariate Index Assay, CA125, modified-American College of Obstetricians and Gynecologists referral guidelines, and clinical assessment among patients undergoing surgery for an adnexal mass after initial evaluation by nongynecologic oncologists. Overall, 770 patients were enrolled by nongynecologic oncologists from 2 related, multiinstitutional, prospective trials and analyzed retrospectively. All patients had preoperative imaging and biomarker analysis. The subset of patients enrolled by nongynecologic oncologists was analyzed to determine the projected referral patterns and sensitivity for malignancy based on multivariate index assay (MIA), CA125, modified-American College of Obstetricians and Gynecologists (ACOG) guidelines, and clinical assessment compared with actual practice. The prevalence of malignancy was 21.3% (n = 164). In clinical practice, 462/770 patients (60.0%) were referred to a gynecologic oncologist for surgery. Triage based on CA125 predicted referral of 157/770 patients (20.4%) with sensitivity of 68.3% (95% confidence interval [CI], 60.8-74.9). Triage based on modified-ACOG guidelines would have resulted in referral of 256/770 patients (33.2%) with a sensitivity of 79.3% (95% CI, 72.4-84.8). Clinical assessment predicted referral of 184/763 patients (24.1%) with a sensitivity of 73.2% (95% CI, 65.9-79.4). Risk stratification using multivariate index assay would have resulted in referral of 429/770 (55.7%) patients, with sensitivity of 90.2% (95% CI, 84.7-93.9). MIA demonstrated statistically
significant higher sensitivity ($P < .0001$) and lower specificity ($P < .0001$) for detecting malignancy compared with clinical assessment, CA125, and modified-ACOG guidelines.

Goodrich, et al. (2014) investigated the relationship between imaging and the multivariate index assay (MIA) in the prediction of the likelihood of ovarian malignancy before surgery. Subjects were recruited in 2 related prospective, multiinstitutional trials that involved 44 sites across the United States. Women had ovarian imaging, biomarker analysis, and surgery for an adnexal mass. Ovarian tumors were classified as high risk for solid or papillary morphologic condition on imaging study. Biomarker and imaging results were correlated with surgical findings. Of the 1110 women who were enrolled with an adnexal mass on imaging, 1024 cases were evaluable. There were 255 malignant and 769 benign tumors. High-risk findings were present in 46% of 1232 imaging tests and 61% of 1024 MIA tests. The risk of malignancy increased with rising MIA scores; similarly, the likelihood of malignancy was higher for high-risk, compared with low-risk, imaging. Sensitivity and specificity for the prediction of malignancy were 98% (95% CI, 92-99) and 31% (95% CI, 27-34) for ultrasound or MIA; 68% (95% CI, 58-77) and 75% (95% CI, 72-78) for ultrasound and MIA, respectively. For computed tomography scan or MIA, sensitivity was 97% (95% CI, 92-99) and specificity was 22% (95% CI, 16-28); the sensitivity and specificity for computed tomography scan and MIA were 71% (95% CI, 62-79) and 70% (95% CI, 63-76). Only 1.6% of ovarian tumors were malignant when both tests indicated low risk.

An assessment by the BlueCross BlueShield Association Technology Evaluation Center (BCBSA, 2013) stated: "The evidence regarding the effect of OVA1 ...on health outcomes is indirect and based on studies of diagnostic performance of the tests in patients undergoing surgery for adnexal masses. Although the studies show improvements in sensitivity and worsening of specificity with the use of the tests in conjunction with clinical assessment, there are problems in concluding that this results in improved health outcomes. The clinical assessment performed in the studies is not well characterized. Although OVA1 improves sensitivity, specificity declines so much that most patients test
positive."

An technology assessment by the ECRI Institute (2015) concluded that the evidence on OVA1 consists of cross-sectional diagnostic accuracy studies. This evidence as reported in article abstracts is unclear as to whether use of OVA1 improves patient-oriented outcomes because none of the studies reported the direct impact of these tests on survival or quality of life. The primary rationale for using these tests is to select the type of surgeon to perform the primary surgery.

Stewart, et al. (2016) reported on a survey of primary care physicians on how often they refer patients diagnosed with ovarian cancer to gynecological oncologists, finding that a total of 84% of primary care physicians (87% of family/general practitioners, 81% of internists and obstetrician/gynecologists) said they always referred patients to gynecologic oncologists for treatment. Common reasons for not always referring were patient preference or lack of gynecologic oncologists in the practice area. A total of 23% of primary care physicians had heard of the OVA1 test, which helps to determine whether gynecologic oncologist referral is needed. The authors noted that, although referral rates reported here are high, it is not clear whether ovarian cancer patients are actually seeing gynecologic oncologists for care.

Eskander, et al. (2016) conducted a retrospective chart review of patients who received the OVA1. Twenty-two obstetricians/gynecologists were recruited from a variety of practices and hospitals throughout the United States. A total of 136 patients with elevated-risk assay results were assessed, of whom 122 underwent surgery to remove an adnexal mass. Prior to surgery, 98 (80%) of the patients were referred to a gynecologic oncologist with an additional 11 (9%) having a gynecologic oncologist available if required by intra-operative findings. Primary ovarian cancer was found in 65 (53%) patients, and gynecologic oncologists performed 61 (94%) of the initial surgeries these patients. Similar results were found in premenopausal and postmenopausal patients.
Forde, et al. (2016) conducted an economic analysis model to evaluate the clinical and cost implications of adopting OVA1 in clinical practice versus the modified ACOG referral guidelines and CA-125 alone, over a lifetime horizon, from the perspective of the public payer. Clinical parameters used to characterize patients' disease status, quality of life, and treatment decisions were estimated using the results of published studies; costs were approximated using reimbursement rates from CMS fee schedules. Model endpoints included overall survival (OS), costs, quality-adjusted life years (QALYs), and incremental cost-effectiveness ratio (ICER). The cost-effectiveness threshold was set to $50,000 per QALY. One-way sensitivity analysis was performed to assess uncertainty of individual parameters included in the analysis. All costs were reported in 2014 US dollars. Use of OVA1 was cost-effective, resulting in fewer re-operations and pre-treatment CT scans. Overall OVA1 resulted in an ICER of $35,094/QALY gained. OVA1 was also cost-saving and QALY-increasing compared to use of CA-125 alone with an ICER of $12,189/QALY gained. One-way sensitivity analysis showed the ICER was most affected by the following parameters: (1) sensitivity of OVA1; (2) sensitivity of mACOG; and (3) percentage of patients, not referred to a gynecologic oncologist, who were correctly diagnosed with advanced epithelial ovarian cancer (EOC). The authors concluded that OVA1 is a more cost-effective triage strategy than mACOG or CA-125. It is expected to increase the percentage of women with ovarian cancer that are referred to gynecologic oncologists, which is shown to improve clinical outcomes. Limitations include the use of assumptions when published data was unavailable, and the use of multiple sources for survival data.

Urban, et al. (2017) reported that the addition of a patient-reported symptom index (SI), which captures subjective symptoms in an objective manner, improved the sensitivity of the OVA1 multivariate index assay (MIA). The investigators conducted a prospective study of patients seen at a tertiary care medical center. Following consent, patients completed an SI and preoperative serum was collected for an OVA1 multivariate index assay. Results for the SI and OVA1 were correlated with operative findings and surgical pathology. Of 218 patients enrolled, 124
(56.9%) had benign disease and 94 (43.1%) had borderline tumors or carcinomas. Sixty-six patients had a primary ovarian or fallopian tube cancer. The median age of patients enrolled in this study was 54 years (interquartile range, 44-63 years), of whom 148 (67.9%) were postmenopausal. More than a third (36.3%) of patients with benign masses was accurately identified as low risk by MIA and SI. The sensitivity, specificity, positive predictive value and negative predictive value (NPV) of the SI relative to primary ovarian cancer was 87.9% (95% CI, 77.9%-93.7%), 70.2% (95% CI, 61.6%-77.5%), 61.1% (95% CI 51.0-70.2%) and 91.6% (95% CI, 84.3%-95.7%), respectively. The sensitivity, specificity, PPV and NPV of CA125 was 75.4% (95% CI, 63.7%-84.2%), 85.7% (95% CI 78.3%-90.9%), 74.2% (62.6%-82.3%) and 86.4% (95% CI, 79.1%-91.5%), respectively. The sensitivity, specificity, PPV and NPV of the MIA were 93.9% (95% CI, 85.4%-97.6%), 55.6 (95% CI 46.9%-64.1%), 53.0% (95% CI 44.0%-61.8% and 94.5% (95% CI, 94.5%-100%), respectively. The overall sensitivity for the combination of MIA plus SI was 100% (66/66; 95% CI, 94.5%-100%), and specificity was 36.3% (45/124; 95% CI, 28.4%-45.0%), with a PPV of 45.5% (37.6% to 53.6%) and a NPV of 100% (95% CI, 92.1%-100%). Limitations of this study noted by the authors include the small sample size and the high prevalence of ovarian malignancies in this population that was largely from a tertiary care center. It should also be noted that the sensitivity and negative predictive value of SI plus CA 125 was 96.9% (95% CI 89.5%-99.2%) and 97.3% (95% CI 90.5%-99.2%), which exceeded that of MIA alone but was somewhat less than MIA plus SI.

Ovarian cancer guidelines from the National Comprehensive Cancer Network (2016) note that the Society of Gynecologic Oncology (SGO), the FDA, and the Mayo Clinic have stated that the OVA1 test should not be used as a screening tool to detect ovarian cancer. The NCCN explains that the OVA1 attempts to preoperatively classify adnexal masses as benign or malignant and suggests that patients can be assessed for who should undergo surgery by an experienced gynecologic oncologist and who can have surgery in the community. "Based upon data documenting an increased survival, NCCN guidelines panel members recommended that all patients should undergo surgery by an experienced gynecologic oncologist (Category 1
Guidelines on management of adnexal masses from the American College of Obstetricians and Gynecologists (ACOG, 2016) state that the OVA1 multivariate index assay has demonstrated higher sensitivity and negative predictive value compared with clinical impression and CA 125 alone. The guidelines state that serum biomarker panels [OVA1 and ROMA] may be used as an alternative to CA 125 alone in determining the need for referral to or consultation with a gynecological oncologist when an adnexal mass requires surgery. The guidelines state that trials that have evaluated the predictive value of these panels show potential for improved specificity; "[h]owever, comparative research has not yet defined the best testing approach."

ACOG guidelines (2016) state that, primarily based upon consensus and expert opinion (Level C), "[s]erum biomarker panels may be used as an alternative to CA 125 level alone in determining the need for referral to or consultation with a gynecological oncologist when an adnexal mass requires surgery." The guidelines state that, based upon "limited or inconsistent" evidence (Level B), consultation or referral to a gynecological oncologist is recommended for women with an adnexal mass that meet one or more of the following criteria; 1) postmenopausal with elevated CA 125 level, ultrasound findings suggestive of malignancy, ascites, a nodular or fixed pelvic mass, or evidence of abdominal or distant metastases; 2) premenopausal with very elevated CA 125 level, ultrasound findings suggestive of malignancy, ascites, a nodular or fixed pelvic mass, or evidence of abdominal or distant metastases; 3) premenopausal or postmenopausal with an elevated score on a formal risk assessment test such as the multivariate index assay, risk of malignancy index, or the Risk of Ovarian Malignancy algorithm or one of the ultrasound-based scoring systems from the International Ovarian Tumor Analysis group.

The UK National Institute for Health Research Health Technology Assessment Programme has commissioned an assessment (Westwood, et al., 2016) comparing the Risk of Malignancy Index (RMI) to alternative risk scores for ovarian cancer, including
Overa/OVA2 (Vermillion), as well as the ROMA score, simple rules ultrasound classification system (IOTA), Assessment of Different NEoplasias in the adnexa (ADNEX) model (IOTA group). The assessment is scheduled to be completed in 2017.

**ColonSentry**

The ColonSentry test (GeneNews, Toronto, Canada) measures the expression of seven genes, which serve as biomarkers to detect colorectal cancer. Interpretation of the status of these seven biomarkers is intended to assist physicians in identifying patients who have an increased current risk. According to the manufacturer, individuals assessed as having an increased current risk of colorectal cancer should consider having a colonoscopy. Individuals assessed as having a decreased current risk of colorectal cancer should discuss with their doctor further screening, including repeating ColonSentry at regular intervals. There is a lack of evidence in the peer-reviewed published medical literature on the effectiveness of colorectal cancer screening with ColonSentry. No current evidence-based guidelines from medical professional organizations or public health agencies recommend ColonSentry for colorectal cancer screening.

**Prostate Px**

Prostate Px (Aureon) uses a prostate cancer patient's biopsy tissue to provide an assessment of disease severity and disease recurrence. Clinical data is integrated with an analysis of each patient’s cancer using tissue histology and molecular biomarkers, such as androgen receptor, associated with disease progression. Although the manufacturer states that the results of the Prostate Px can be used in decision-making, there is a lack of evidence of the clinical utility of this test in altering the management of patients such that clinical outcomes are improved.

**Post-Op Px**

Post-Op Px is a prognostic test that utilizes a patented systems pathology approach to analyze prostatectomy tissue by
combining cellular, molecular and clinical information to provide a thorough and more accurate picture of each patient’s individual risk of prostate cancer recurrence. (Aureon, 2010). Donovan et al (2011) evaluated the performance of a systems-based risk assessment tool with standard defined risk groups and the 10 year postoperative normogram for predicting disease progression. The systems model was found to be more accurate than standard risk groups both to predict significant disease progression ($p < 0.001$) and for predicting prostate-specific antigen recurrence ($p < 0.001$). However, this study has not been replicated in the peer-reviewed literature.

**EGFR**

An assessment by the BlueCross BlueShield Association Technology Evaluation Center (2010) concluded that tumor-cell epidermal growth factor receptor (EGFR) mutation analysis to predict response to erlotinib (Tarceva) in patients with advanced non-small cell lung cancer (NSCLC) meets the Blue Cross and Blue Shield Association Technology Evaluation Center (TEC) criteria. Furthermore, guidelines from the National Comprehensive Cancer Network (NCCN, 2010) recommend EGFR testing for the following histologic subtypes of NSCLC: (i) adenocarcinoma, (ii) large cell, and (iii) NSCLC not otherwise specified. Epidermal growth factor receptor testing is not recommended for squamous cell carcinoma.

The Alberta Provincial Thoracic Tumour Team’s clinical practice guideline on “Non-small cell lung cancer stage IV” (2011) stated that “First-line monotherapy with the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor gefitinib is recommended for patients with EGFR mutation-positive NSCLC. Testing for EGFR mutations should take place for all eligible patients with advanced NSCLC and adenocarcinoma histology who are being considered for first-line therapy with gefitinib, irrespective of their gender, ethnicity, and smoking status”.

NCCN non-small cell lung cancer guidelines (2015) state that EGFR and ALK testing should be conducted as part of a multiplex/next generation sequencing. The NCCN NSCLC
Guidelines Panel "strongly endorses broader molecular profiling with the goal of identifying rare mutations for which effective drugs may already be available, or to appropriately counsel patients regarding the availability of clinical trials. Broad molecular profiling is a key component of the improvement of care of patients with NSCLC."

Gao et al (2012) stated that gefitinib and erlotinib are 2 similar small molecules of selective and reversible epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs), which have been approved for second-line or third-line indication in previously treated advanced NSCLC patients. The results of comparing the EGFR-TKI with standard platinum-based doublet chemotherapy as the first-line treatment in advanced NSCLC patients with activated EGFR mutation were still controversial. A meta-analysis was performed to derive a more precise estimation of these regimens. Finally, 6 eligible trials involved 1,021 patients were identified. The patients receiving EGFR-TKI as front-line therapy had a significantly longer PFS than patients treated with chemotherapy [median PFS was 9.5 versus 5.9 months; HR = 0.37; 95 % CI: 0.27 to 0.52; p < 0.001]. The overall response rate (ORR) of EGFR-TKI was 66.60 %, whereas the ORR of chemotherapy regimen was 30.62 %, which was also a statistically significant favor for EGFR-TKI [relative risk (RR) = 5.68; 95 % CI: 3.17 to 10.18; p < 0.001]. The OS was numerically longer in the patients received EGFR-TKI than patients treated by chemotherapy, although the difference did not reach a statistical significance (median OS was 30.5 versus 23.6 months; HR = 0.94; 95 % CI: 0.77 to 1.15; p = 0.57). Comparing with first-line chemotherapy, treatment of EGFR-TKI achieved a statistical significantly longer PFS, higher ORR and numerically longer OS in the advanced NSCLC patients harboring activated EGFR mutations, thus, it should be the first choice in the previously untreated NSCLC patients with activated EGFR mutation.

Guidelines from the American Society for Clinical Oncology (2016) state: "The clinician should not use HER1/epidermal growth factor receptor expression by IHC to guide adjuvant chemotherapy selection" in breast cancer.
Messick et al (2010) evaluated carcinoembryonic antigen cellular adhesion molecule-7 (CEACAM-7) expression in rectal cancer as a predictive recurrence factor. A single-institution colorectal cancer database and a frozen tissue biobank were queried for rectal cancer patients. CEACAM-7 messenger RNA (mRNA) expression from normal rectal mucosa and rectal cancers was analyzed using quantitative real-time polymerase chain reaction (PCR). Expression-level differences among normal tissue, disease-free survivors, and those that developed recurrence were analyzed. A total of 84 patients were included in the study, which consisted of 37 patients with non-recurrent disease (median follow-up of 170 months), 29 patients with recurrent disease, and 18 patients with stage IV disease. CEACAM-7 expression was decreased 21-fold in rectal cancers compared with normal mucosa (p = 0.002). The expression levels of CEACAM-7 were relatively decreased in tumors that developed recurrence compared with non-recurrence, significantly for stage II patients (14-fold relative decrease, p = 0.002). For stages I-III, disease-free survival segregates were based on relative CEACAM-7 expression values (p = 0.036), specifically for stage II (p = 0.018). The authors concluded that CEACAM-7 expression is significantly decreased in rectal cancer. Expression differences between long-term survivors and those with recurrent disease introduce a potential tumor marker to define a subset of patients who benefit most from adjuvant therapy. Moreover, they stated that additional study and validation are needed before CEACAM-7 can be applied in clinical settings.

CFL1

Castro et al (2010) assessed the potential value of cofilin (CFL1) gene (main member of the invasion/metastasis pathway) as a prognostic and predictive NSCLC biomarker. Meta-analysis of tumor tissue microarray was applied to examine expression of CFL1 in archival lung cancer samples from 111 patients, and its clinicopathologic significance was investigated. The robustness of the finding was validated using another independent data set. Finally, the authors assayed in vitro the role of CFL1 levels in
tumor invasiveness and drug resistance using 6 human NSCLC cell lines with different basal degrees of CFL1 gene expression. Cofilin levels in biopsies discriminated between good and bad prognosis at early tumor stages (IA, IB, and IIA/B), where high CFL1 levels are correlated with lower overall survival rate (p < 0.0001). Biomarker performance was further analyzed by IHC, hazard ratio (p < 0.001), and receiver-operating characteristic curve (area = 0.787; p < 0.001). High CFL1 mRNA levels and protein content are positively correlated with cellular invasiveness (determined by Matrigel Invasion Chamber System) and resistance (2-fold increase in drug 50 % growth inhibition dose) against a list of 22 alkylating agents. Hierarchical clustering analysis of the CFL1 gene network had the same robustness for stratified NSCLC patients. The authors concluded that these findings indicated that the CFL1 gene and its functional gene network can be used as prognostic biomarkers for NSCLC and could also guide chemotherapeutic interventions. Moreover, prospective, large-scale, randomized clinical trials are needed to establish the role of CFL1 as a prognostic and drug resistance marker for NSCLC.

EarlyCDT-Lung

The EarlyCDT-Lung (Oncimmune, De Soto, KS) test measures antibodies to 6 tumor-associated antigens: p53, NY-ESO-1, CAGE, GBU4-5, Annexin 1, and SOX2. Elevation of any one of the panel of immuno-biomarkers above a predetermined cut-off value suggests that a tumor might be present. The test is designed to be used in conjunction with diagnostic imaging. High-risk individuals with a positive EarlyCDT-Lung would have additional testing such as a CT scan or the test would be used as a follow-up test for indeterminate lung nodules identified by CT.

Boyle et al (2011) reported the sensitivity and specificity of an autoantibody panel of 6 tumor-related antigens (p53, NY-ESO-1, CAGE, GBU4-5, Annexin 1 and SOX2) in patients with lung cancer. Three cohorts of patients with newly diagnosed lung cancer were identified: group 1 (n = 145), group 2 (n = 241) and group 3 (n = 269). Patients were individually matched by gender, age and smoking history to a control individual with no history of malignant disease. Serum samples were obtained after diagnosis
but before any anticancer treatment. Autoantibody levels were measured against the panel of 6 tumor-related antigens (p53, NY-ESO-1, CAGE, GBU4-5, Annexin 1 and SOX2). Assay sensitivity was tested in relation to demographic variables and cancer type/stage. The autoantibody panel demonstrated a sensitivity/specificity of 36 %/91 %, 39 %/89 % and 37 %/90 % in groups 1, 2 and 3, respectively, with good reproducibility. There was no significant difference between different lung cancer stages, indicating that the antigens included covered the different types of lung cancer well. The authors concluded that the assay confirms the value of an autoantibody panel as a diagnostic tool and offers a potential system for monitoring patients at high-risk of lung cancer.

There is insufficient evidence of the effectiveness of the EarlyCDT-Lung as a screening test for the early detection of lung cancer. Systematic screening for lung cancer is not unequivocally recommended by any major professional organization. The USPSTF (2004) concluded that current evidence was insufficient to recommend for, or against, screening for lung cancer. Whether earlier detection of lung cancer will translate to a mortality benefit remains unclear.

E-cad

Deeb et al (2004) stated that E-cadherin (E-cad) and epidermal growth factor receptor (EGFR) are important cell adhesion and signaling pathway mediators. They reported the results of a study which aimed to assess their expression in lung adenocarcinoma (AdC) and squamous cell carcinoma (SCC) and their association with clinicopathologic variables. Two to three cores from 130 resectable lung cancers (stages I-IIIA) were arrayed into three blocks using a Beecher system. Markers expression and coexpression were analyzed against clinicopathologic variables (age, gender, smoking status, performance status, weight loss, histology, grade, stage, and lymph node involvement) and patient survival. For E-cad, 65 cases (55%) were positive (+), 53 (45%) were negative (-); and for EGFR, 43 cases (34%) were (+), and 83 (66%) were (-). There was no significant association between E-cad or EGFR, and any of the
clinicopathologic variables except for an association between EGFR(+) and SCC histologic type. Both negative and cytoplasmic staining of E-cad correlated with shorter patient survival with P=0.008 and 0.002, respectively. EGFR expression did not correlate with patient survival, but, patients with E-cad(-)/EGFR(+) phenotype had poorer survival than those with E-cad(+)/EGFR(-) (P=0.026). The authors concluded that lung AdC and SCC may be stratified based on expression of E-cad and EGFR with the E-cad(-)/EGFR(+) expression having a worse disease outcome.

EML4-ALK

Yoshida et al (2011) report that a subset of lung cancers harbors an EML4-ALK (echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase) gene fusion, and they examined 15 lung adenocarcinomas with reverse-transcriptase polymerase chain reaction-proven EML4-ALK fusion transcripts and 30 ALK-negative cases. Positive rearrangement signals (splits or isolated 3' signals) were identified in 13 to 78% (mean ± SD, 41% ± 19%) of tumor cells in the ALK-positive cohort and in 0 to 15% (mean ± SD, 6% ± 4%) of cells in the ALK-negative cohort. Sensitivity was at 93% and specificity at 100%. The only false-negative tumor having only 13% CISH-positive cells displayed predominantly (76%) isolated 5' signals unaccompanied by 3' signals. FISH showed largely similar signal profiles, and the results were completely concordant with CISH. The authors stated that they have successfully introduced CISH for diagnosing EML4-ALK-positive lung adenocarcinoma. This method allows simultaneous visualization of genetics and tumor cytomorphology and facilitates the molecular evaluation and could be applicable in clinical practice to detect lung cancer that may be responsive to ALK inhibitors.

Ellis et al (2011) conducted a systematic review and a consensus meeting of Canadian lung cancer oncologists and pathologists to make recommendations on the use of biomarkers in NSCLC. The articles were reviewed by pairs of oncologists and pathologists to determine eligibility for inclusion. Ten oncologists and pathologists reviewed and summarized the literature at a meeting attended by 37 individuals. The findings included that there is
some evidence that histology is prognostic for survival as well as evidence from multiple randomized clinical trials to recommend the following: histologic subtype is predictive of treatment efficacy and for some agents toxicity. Immunohistochemistry testing should be performed on NSCLC specimens that cannot be classified accurately with conventional H&E staining. As EGFR mutations are predictive of benefit from tyrosine kinase inhibitors, diagnostic NSCLC samples should be routinely tested for EGFR-activating mutations. Clinical data on K-RAS mutations are inconsistent, therefore testing is not recommended. There is insufficient evidence to recommend other biomarker testing. No biomarkers to date reliably predict improved efficacy for anti-VEGF therapy. The authors concluded that routine assessment for EML4/ALK mutations is not recommended at present, although emerging data suggest that it may become valuable in the near future.

**MUC4**

Shanmugan and co-workers (2010) stated that mucin 4 (MUC4) is aberrantly expressed in colorectal adenocarcinomas (CRCs) but its prognostic value is unknown. Archival tissue specimens collected from 132 CRC patients who underwent surgical resection without pre-surgery or post-surgery therapy were evaluated for expression of MUC4 by using a mouse monoclonal antibody and horseradish peroxidase. MUC4 expression levels were correlated with clinicopathologic features and patient survival. Survival was estimated by both uni-variate Kaplan-Meier and multi-variate Cox regression methods. In both normal colonic epithelium and CRCs, MUC4 staining was localized primarily in the cytoplasm. The optimal immunostaining cut-off value (greater than or equal to 75 % positive cells and an immunostaining score greater than or equal to 2.0), which was derived by using the bootstrap method, was used to categorize CRCs into groups of high expression (33 of 132 patients; 25 %) or low expression (99 of 132 patients; 75 %). Patients who had early stage tumors (stages I and II) with high MUC4 expression had a shorter disease-specific survival (log-rank; p = 0.007) than patients who had with low expression. Patients who had advanced-stage CRCs (stages III and IV) did not demonstrate such a difference (log-rank; p = 0.108). Multi-variate
regression models that were generated separately for patients with early stage and advanced-stage CRC confirmed that increased expression of MUC4 was an independent indicator of a poor prognosis only for patients who had early stage CRCs (HR 3.77; 95 % CI: 1.46 to 9.73). The authors stated that after validating these findings in larger retrospective and prospective studies, a stage-based analyses could establish the utility of MUC4 as a prognostic molecular marker of early stage CRC.

ProOnc TumorSourceDx

ProOnc TumorSourceDx test is designed to identify tissue or origin for metastatic tumor. It identifies 25 possible classes of tissue origin corresponding to 17 distinct tissues and organs. It requires only 48 microRNAs to identify tissue of origin based on microRNA expression levels. However, there is insufficient evidence regarding its clinical value as tumor markers.

SAA

Cocco and associates (2010) examined the expression of serum amyloid A (SAA) in endometrial endometrioid carcinoma and evaluated its potential as a serum biomarker. SAA gene and protein expression levels were evaluated in endometrial endometrioid carcinoma and normal endometrial tissues, by real-time PCR, IHC, and flow cytometry. SAA concentration in 194 serum samples from 50 healthy women, 42 women with benign diseases, and 102 patients including 49 grade 1, 38 grade 2, and 15 grade 3 endometrial endometrioid carcinoma was also studied by a sensitive bead-based immunoassay. SAA gene expression levels were significantly higher in endometrial endometrioid carcinoma when compared with normal endometrial tissues (mean copy number by real-time PCR = 182 versus 1.9; p = 0.001). IHC revealed diffuse cytoplasmic SAA protein staining in poorly differentiated endometrial endometrioid carcinoma tissues. High intra-cellular levels of SAA were identified in primary endometrial endometrioid carcinoma cell lines evaluated by flow cytometry, and SAA was found to be actively secreted in vitro. SAA concentrations (microg/ml) had medians of 6.0 in normal healthy women and 6.0 in patients with benign disease.
In contrast, SAA values in the serum of endometrial endometrioid carcinoma patients had a median of 23.7, significantly higher than those of the healthy group (p = 0.001) and benign group (p = 0.001). Patients harboring G3 endometrial endometrioid carcinoma were found to have SAA concentrations significantly higher than those of G1/G2 patients. The authors concluded that SAA is not only a liver-secreted protein, but is also an endometrial endometrioid carcinoma cell product. SAA is expressed and actively secreted by G3 endometrial endometrioid carcinoma, and it is present in high concentration in the serum of endometrial endometrioid carcinoma patients. SAA may represent a novel biomarker for endometrial endometrioid carcinoma to monitor disease recurrence and response to therapy. They stated that additional studies are needed to validate these findings.

Caris Target Now / Caris Molecular Profiling Service

Molecular Intelligence Services (formerly Target Now Molecular Profiling Test) uses a multi-platform profiling approach including gene sequencing (NGS and Sanger), protein expression analysis (immunohistochemistry) and gene copy number analysis (chromogenic or fluorescence in situ hybridization [FISH]). The test has been used to examine tumor samples for underlying molecular alterations that may yield insights into potentially overlapping and different therapeutic options for individuals with these tumor types.

According to the manufacturer, the Caris Life Sciences molecular profiling test, Caris Target Now, examines the genetic and molecular changes unique to a patient's tumor so that treatment options may be matched to the tumor's molecular profile. The manufacturer states that the Caris Target Now test is performed after a cancer diagnosis has been established and the patient has exhausted standard of care therapies or if questions in therapeutic management exist. Using tumor samples obtained from a biopsy, the tumor is examined to identify biomarkers that may have an influence on therapy. Using this information, Caris Target Now is intended to provide information on the drugs that will be more likely to produce a positive response.
manufacturer states that Caris Target Now can be used with any solid cancer such as lung cancer, breast cancer, and prostate cancer.

There is insufficient evidence to support the use of Caris Target Now molecular profiling. A study (Von Hoff et al, 2010) compared the progression-free survival (PFS) of patients with refractory metastatic cancers using a treatment regimen selected by Caris Target Now molecular profiling of the patient's tumor with the PFS for the most recent regimen on which the patient had experienced progression. The investigators prespecified that a molecular profiling approach would be deemed of clinical benefit for the individual patient who had a PFS ratio (defined as a ratio of PFS on molecular profiling-selected therapy to PFS on prior therapy) of greater than or equal to 1.3. In 86 patients who had molecular profiling attempted, there was a molecular target detected in 84 (98 %). Sixty-six of the 84 patients were treated according to molecular profiling results. Eighteen (27 %) of 66 patients had a PFS ratio of greater than or equal 1.3 (95 % CI:17 % to 38 %). Therefore, the null hypothesis (that less than or equal to 15 % of this patient population would have a PFS ratio of greater than or equal to 1.3) was rejected. The authors concluded that, in 27 % of patients, the molecular profiling approach resulted in a longer PFS on an molecular profiling-suggested regimen than on the regimen on which the patient had just experienced progression. An accompanying editorial (Doroshow, 2010) noted that the trial had a number of significant limitations, including uncertainty surrounding the achievement of time to progression (the study’s primary endpoint), and a lack of a randomized design for this trial.

A report by the National Horizon Scanning Centre (2013) stated that the company stated that the tumor profiling service provided by Caris Life Sciences has been extensively altered with the addition of several new technologies. The new service is named Caris Life Sciences Molecular Intelligence Services. The NHSC stated that randomized controlled trials comparing clinical outcomes for patients using Caris molecular profiling to those receiving standard specialist care are needed to determine whether this testing service is effective and cost-effective.
CoA racemase (P504S) and HMWCK (34betaE12)

Kumaresan et al (2010) reviewed 1034 cases of morphologically difficult prostate cancer, which were divided into benign (585), malignant (399) and suspicious (50) and evaluated using CoA racemase (P504S) and HMWCK (34betaE12). Forty nine suspicious cases were resolved by using both markers whereas 1 case was resolved by further support with CD68. The original diagnosis was changed in 15 of 50 suspicious cases from benign to malignant, one case from benign to high grade PIN, and in one case from malignant to benign. The authors concluded that a combination of HMWCK and AMACR is of value in combating morphologically suspicious cases and that although the sensitivity and specificity of HMWCK and AMACR in this study were high, “it should be used with caution, keeping in mind all their pitfalls and limitations.”

P504S

Murray et al (2010) studied P504S expressing circulating prostate cells as a marker for prostate cancer. The authors stated that PSA is the only biomarker routinely used in screening. This study aimed to develop a system to test the presence of circulating prostate cells in men without a diagnosis of prostate cancer in relation with age, serum PSA levels and prostate biopsy by determining the co-expression of several markers such as CD82, HER-2 and matrix metalloproteinase 2 MMP-20. The results indicated that among 409 men screened for prostate cancer 16.6% were positive for circulating prostate cells. The authors concluded that the study of circulating prostate cells with various markers could be a useful complementary screening test for prostate cancer in men with increased PSA level.

FLT3

FLT3 has been used to predict prognosis in acute myelogenous leukemia (Chin, et al, 2006). Mutations in FLT3 are common in AML and have been associated with poorer survival in children and in younger adults with normal cytogenetics receiving intensive chemotherapy.
The NCCN Task Force issued a report in November of 2011 which updated their position regarding molecular markers for diagnosis, prognosis, prediction, and companion diagnostic markers (Febbo et al., 2011). As a result of these recommendations, use of MGMT, IDH mutation and 1p/19q codeletion are now established for glioma. Also, use of ALK gene fusion has been established for non-small cell lung cancer. The updated NCCN guidelines have not yet established the efficacy of ColoPrint, CIMP, LINE-1 hypomethylation, or Immune cells for colon cancer. Similarly, the efficacy of FLT3-TKD mutation, WT1 mutation, RUNX1 mutation, MLL-PTD, IDH1 mutation, IDH2 R172, and IDH2 codon 140 mutation has not been established for use in acute myeloid leukemia.

**ColoPrint**

ColoPrint (Agendia) is an 18-gene profile that classifies colon cancer into Low Risk or High Risk of relapse, by measuring genes representative of the metastatic pathways of colon cancer metastases which were selected for their predictive relationship to 5-year distant metastases probability (Raman, et al., 2013). ColoPrint is indicated for stage II colon cancer, and provides relapse risk stratification independent of clinical and pathologic factors such as T4-stage and MSI status. ColoPrint determines if the patient is a candidate for chemotherapy. An NCCN Task Force report (NCCN, 2011) concluded that the efficacy of ColoPrint has not been established.

**DecisionDx**

The DecisionDx test is a gene expression profile that determines the molecular signature of a patient's melanoma. The results of the test provide knowledge regarding the risk of near term metastasis (5 years). Tumors with a Class 1 signature are associated with a good prognosis and a low potential to spread (or metastasize), while tumors with a Class 2 signature have a high potential to spread. There is a lack of adequate published evidence on the clinical utility of this test. Current guidelines on melanoma from the National Comprehensive Cancer Network make no recommendation for this test.
Aaberg, et al. (2014) conducted a chart review and cross-sectional survey of ophthalmologists who treat uveal melanoma to assess current clinical practices for uveal melanoma (UM) and the impact of molecular prognostic testing on treatment decisions. This study involved a chart review of all Medicare beneficiaries tested by UM gene expression profile in 2012, conducted under an institutional review board-approved protocol. In addition, 109 ophthalmologists specializing in the treatment of UM were invited to participate in 24-question survey in 2012; 72 were invited to participate in a 23-question survey in 2014. The review of Medicare medical records included 191 evaluable patients, 88 (46%) with documented medical treatment actions or institutional policies related to surveillance plans. Of these 88, all gene expression profiling (GEP) Class 1 UM patients were treated with low-intensity surveillance. All GEP Class 2 UM patients were treated with high-intensity surveillance (P<0.0001 versus Class 1). There were 36 (19%) with information concerning referrals after initial diagnosis. Of these 36, all 23 Class 2 patients were referred to medical oncology; however, none of the 13 Class 1 patients were referred (P<0.0001 versus Class 1). Only Class 2 patients were recommended for adjunctive treatment regimens. 2012 survey: 50 respondents with an annual median of 35 new UM patients. The majority of respondents (82%) performed molecular analysis of UM tumors after fine needle biopsy (FNAB); median: 15 FNAB per year; 2014 survey: 35 respondents with an annual median of 30 new UM patients. The majority offered molecular analyses of UM tumor samples to most patients. Patients with low metastatic risk (disomy 3 or GEP Class 1) were generally assigned to less frequent (every 6 or 12 months) and less intensive clinical visits. Patients with high metastatic risk (monosomy 3 or GEP Class 2) were assigned to more frequent surveillance with hepatic imaging and liver function testing every 3-6 months. High-risk patients were considered more suitable for adjuvant treatment protocols.

Chappell, et al. (2012) reported on a retrospective case series of uveal melanoma patients gene expression profiles to characterize the clinical spectrum of class 1 and class 2 uveal melanomas and their relationship with intraocular proton radiation response. A total of 197 uveal melanoma patients from a single institution
were analyzed for pathology, clinical characteristics, and response to radiation therapy. A total of 126 patients (64%) had class 1 tumors and 71 (36%) had class 2 tumors. Patients with class 2 tumors had more advanced age (mean: 64 years vs 57 years; \( P = .001 \)), had thicker initial mean ultrasound measurements (7.4 mm vs 5.9 mm; \( P = .0007 \)), and were more likely to have epithelioid or mixed cells on cytopathology (66% vs 38%; \( P = .0004 \)). Although mean pretreatment and posttreatment ultrasound thicknesses were significantly different between class 1 and class 2 tumors, there was no difference in the mean change in thickness 24 months after radiation therapy (mean difference: class 1 = -1.64 mm, class 2 = -1.47; \( P = .47 \)) or in the overall rate of thickness change (slope: \( P = .64 \)). Class 2 tumors were more likely to metastasize and cause death than class 1 tumors (disease-specific surviva [DSS]: \( P < .0001 \)).

Worley, et al. (2007) compared a gene expression-based classifier versus the standard genetic prognostic marker, monosomy 3, for predicting metastasis in uveal melanoma. Gene expression profiling, fluorescence in situ hybridization (FISH), and array comparative genomic hybridization (aCGH) were done on 67 primary uveal melanomas. Clinical and pathologic prognostic factors were also assessed. The investigators found that the gene expression-based molecular classifier assigned 27 tumors to class 1 (low risk) and 25 tumors to class 2 (high risk). By Cox univariate proportional hazards, class 2 signature (\( P = 0.0001 \)), advanced patient age (\( P =0.01 \)), and scleral invasion (\( P = 0.007 \)) were the only variables significantly associated with metastasis. Only the class 2 signature was needed to optimize predictive accuracy in a Cox multivariate model. A less significant association with metastasis was observed for monosomy 3 detected by aCGH (\( P = 0.076 \)) and FISH (\( P = 0.127 \)). The sensitivity and specificity for the molecular classifier (84.6% and 92.9%, respectively) were superior to monosomy 3 detected by aCGH (58.3% and 85.7%, respectively) and FISH (50.0% and 72.7%, respectively). Positive and negative predictive values (91.7% and 86.7%, respectively) and positive and negative likelihood ratios (11.9 and 0.2, respectively) for the molecular classifier were also superior to those for monosomy 3.
In a prospective case series study, Corrêa and Augsburger (2016) sought to determine whether any conventional clinical prognostic factors for metastasis from uveal melanoma retain prognostic significance in multivariate models incorporating gene expression profile (GEP) class of the tumor cells. The investigators conducted a single-institution study of GEP testing and other conventional prognostic factors for metastasis and metastatic death in 299 patients with posterior uveal melanoma evaluated by fine-needle aspiration biopsy (FNAB) at the time of or shortly prior to initial treatment. Univariate prognostic significance of all evaluated potential prognostic variables (patient age, largest linear basal diameter of tumor [LBD], tumor thickness, intraocular location of tumor, melanoma cytomorphologic subtype, and GEP class) was performed by comparison of Kaplan-Meier event rate curves and univariate Cox proportional hazards modeling. Multivariate prognostic significance of combinations of significant prognostic factors identified by univariate analysis was performed using step-up and step-down Cox proportional hazards modeling. GEP class was the strongest prognostic factor for metastatic death in this series. However, tumor LBD, tumor thickness, and intraocular tumor location also proved to be significant individual prognostic factors in this study. On multivariate analysis, a 2-term model that incorporated GEP class and largest basal diameter was associated with strong independent significance of each of the factors.

Corrêa and Augsburger (2014) sought to determine the relative sufficiency of paired aspirates of posterior uveal melanomas obtained by FNAB for cytopathology and GEP, and their prognostic significance for predicting death from metastasis. The investigators conducted a prospective non-randomized single-center study of 159 patients with posterior uveal melanoma sampled by FNAB in at least two tumor sites between September 2007 and December 2010. Cases were analyzed with regard to sufficiency of the obtained aspirates for cytopathologic classification and GEP classification. Statistical strength of associations between variables and GEP class was computed using Chi-square test. Cumulative actuarial survival curves of subgroups of these patients based on their cytopathologic versus GEP-assigned categories were computed by the Kaplan-Meier method. The endpoint for this survival analysis was death from
metastatic uveal melanoma. FNAB aspirates were insufficient for cytopathologic classification in 34 of 159 cases (21.9%). In contrast, FNAB aspirates were insufficient for GEP classification in only one of 159 cases (0.6%). This difference is statistically significant (P < 0.001). Six of 34 tumors (17.6%) that yielded an insufficient aspirate for cytopathologic diagnosis were categorized as GEP class 2, while 43 of 125 tumors (34.7%) that yielded a sufficient aspirate for cytopathologic diagnosis were categorized as GEP class 2. To date, 14 of the 49 patients with a GEP class 2 tumor (28.6%) but only five of the 109 patients with a GEP class 1 tumor (5.6%) have developed metastasis. Fifteen of 125 patients (12%) whose tumors yielded sufficient aspirates for cytopathologic classification but only four of 34 patients (11.8%) whose tumors yielded insufficient aspirates for cytopathologic classification developed metastasis. The median post-biopsy follow-up time for surviving patients in this series was 32.5 months. Cumulative actuarial 5-year probability of death from metastasis 14.1% for those with an insufficient aspirate for cytopathologic classification versus 22.4% for those with a sufficient aspirate for cytopathologic classification (log rank P = 0.68). In contrast, the cumulative actuarial 5-year probability of metastatic death was 8.0% for those with an insufficient/unsatisfactory aspirate for GEP classification or GEP class 1 tumor, versus 45.0% for those with a GEP class 2 tumor (log rank P = 0.005).

In a prospective study, Oniken, et al. (2012) evaluated the prognostic performance of the DecisionDx 15 gene expression profiling (GEP) assay that assigns primary posterior uveal melanomas to prognostic subgroups: class 1 (low metastatic risk) and class 2 (high metastatic risk). A total of 459 patients with posterior uveal melanoma were enrolled from 12 independent centers. Tumors were classified by GEP as class 1 or class 2. The first 260 samples were also analyzed for chromosome 3 status using a single nucleotide polymorphism assay. Net reclassification improvement analysis was performed to compare the prognostic accuracy of GEP with the 7th edition clinical Tumor-Node-Metastasis (TNM) classification and chromosome 3 status. The investigators found that the GEP assay successfully classified 446 of 459 cases (97.2%). The GEP was class 1 in 276 cases (61.9%)
and class 2 in 170 cases (38.1%). Median follow-up was 17.4
months (mean, 18.0 months). Metastasis was detected in 3 class
1 cases (1.1%) and 44 class 2 cases (25.9%) (log-rank test, P<10(-
14)). Although there was an association between GEP class 2 and
monosomy 3 (Fisher exact test, P<0.0001), 54 of 260 tumors
(20.8%) were discordant for GEP and chromosome 3 status,
among which GEP demonstrated superior prognostic accuracy
(log-rank test, P = 0.0001). By using multivariate Cox modeling,
GEP class had a stronger independent association with
metastasis than any other prognostic factor (P<0.0001).
Chromosome 3 status did not contribute additional prognostic
information that was independent of GEP (P = 0.2). At 3 years
follow-up, the net reclassification improvement of GEP over TNM
classification was 0.43 (P = 0.001) and 0.38 (P = 0.004) over
chromosome 3 status.

Klufas et al (2015) reported their experience with uveal
melanoma (UM)-specific GEP testing on a series of choroidal
metastatic tumors confirmed by cytopathology so that clinicians
may be aware that receiving a class 1 or class 2 test result in non-
melanoma is possible. These investigators performed a
retrospective review of all cytopathology and DecisionDx-UM GEP
reports between January 2012 to December 2014 from intra-
operative FNA biopsy of choroidal tumors undergoing
brachytherapy. A total of 4 patients were identified to have
cytopathology consistent with a non-melanoma primary. All 4
patients presented with a unilateral, single choroidal tumor,
which was treated with iodine-125 brachytherapy and underwent
intra-operative FNA biopsy for cytopathology and UM-specific
GEP testing for molecular prognostication. Gene expression
profile testing of the choroidal tumor in each patient revealed
class 1A in 3 patients and class 2 in 1 patient. The authors
concluded that DecisionDx-UM GEP may be a helpful test for
molecular prognostication in patients with UM; however, class 1
and class 2 test results are indeed possible in the setting of a non-
melanoma malignancy. They recommended that cytopathology
and/or other melanoma-specific testing be performed in all cases
of suspected choroidal melanoma because GEP with this assay is
unable to rule out the diagnosis of a choroidal melanoma.
Plasseuard, et al. (2016) sought to evaluate the clinical validity and utility of DecisionDx-UM. Beginning March 2010, 70 patients were enrolled in a prospective, multicenter, IRB-approved study to document patient management differences and clinical outcomes associated with low-risk Class 1 and high-risk Class 2 results indicated by DecisionDx-UM testing. Thirty-seven patients in the prospective study were Class 1 and 33 were Class 2. Class 1 patients had 100% 3-year metastasis-free survival compared to 63% for Class 2 (log rank test \( p = 0.003 \)) with 27.3 median follow-up months in this interim analysis. Class 2 patients received significantly higher-intensity monitoring and more oncology/clinical trial referrals compared to Class 1 patients (Fisher’s exact test \( p = 2.1 \times 10^{-13} \) and \( p = 0.04 \), respectively).

The investigators concluded that the results of this study provide additional, prospective evidence in an independent cohort of patients that Class 1 and Class 2 patients are managed according to the differential metastatic risk indicated by DecisionDx-UM.

In a review of the management of ocular melanoma, Blum, et al. (2016) commented on the potential use of DecisionDx, noting that, although there is no clear survival benefit from earlier detection of metastatic disease, patients could benefit from clinical trial eligibility and palliative therapy with earlier detection.

The United Kingdom’s national guidelines on “Uveal melanoma” (2015) recommended these molecular diagnostic tests be performed as part of a research protocol.

Gerami, et al. (2015) reported on the DecisionDx predictive genetic signature for classifying tumors as class 1 (low risk) or class 2 (high risk) for metastasis. Using earlier studies, they compared differences in the levels of 28 genes, including some control genes, using RT-PCR. The 5-year disease-free survival (DFS) rate for the 164 sample training set was 91% for class 1 and 25% for class 2 (\( P < 0.0001 \)), while the 5-year DFS rate for the 104 sample validation set (stage I–IV) was 97% for class 1 and 31% for class 2 (\( P < 0.0001 \)). The signature was used to classify stage I and stage IIA tumors, accurately predicting 120 of 134 tumors without metastases as class 1 (90%) and 24 of 30 tumors with metastases
Sidiropoulos et al (2014) noted that primary dermal melanoma (PDM) is a subtype of melanoma confined to the dermis that may be morphologically impossible to distinguish from cutaneous metastatic melanoma (CMM). These researchers sought to better characterize PDM by describing the clinical, histologic, and molecular features of 49 cases of PDM and examine if a gene expression-profiling (GEP) test could help distinguish PDM from CMM. They described 49 cases of PDM and examined if any clinical or histopathologic features had a statistically significant relationship with outcome. In addition, these investigators performed a melanoma GEP test on a subset of the PDM and CMM cases. Overall recurrence was infrequent and seen in 9 of 49 cases; 6 patients had loco-regional recurrences and 3 patients had distant metastasis. None of the clinical or histologic parameters showed a statistically significant relationship with recurrence. There was a statistically significant association of a class I signature by DecisionDx-Melanoma assay (Castle Biosciences Inc., Friendswood, TX) for PDM whereas CMM were more frequently class II (p = 0.023). The authors concluded that most conventional staging parameters used for prognosis in cutaneous melanoma have limited applicability to PDM. They stated that the melanoma prognostic assay may be a useful tool for distinguishing PDM from CMM.

Berger, et al. (2016) sought to ascertain clinical management changes determined by DecisionDx-Melanoma outcome, which classifies cutaneous melanoma (CM) patients being at low (Class 1) or high (Class 2) risk for recurrence. Medical charts were reviewed from 156 CM patients from six institutions (three dermatology and three surgical oncology practices) who were consecutively tested between May 2013 and December 2015. Clinical management data that were compiled and compared before and after receipt of the 31-gene expression test result included frequency of physical exams, frequency and modality of imaging, and referrals to surgical and medical oncologists. Forty-two percent of patients were Stage I, 47% were Stage II and 8% were Stage III. Overall, 95 patients (61%) were Class 1 and 61 (39%) were Class 2. Documented changes in management were
observed in 82 (53%) patients, with the majority of Class 2 patients (77%) undergoing management changes compared to 37% of Class 1 patients (p < 0.0001 by Fisher's exact test). The majority (77/82, 94%) of these changes were concordant with the risk indicated by the test result (p < 0.0001 by Fisher's exact test), with increased management intensity for Class 2 patients and reduced management intensity for Class 1 patients. The authors concluded that molecular risk classification by gene expression profiling has clinical impact and influences physicians to direct clinical management of CM patients. The vast majority of the changes implemented after the receipt of test results were reflective of the low or high recurrence risk associated with the patient's molecular classification. Because follow-up data was not collected for this patient cohort, the study is limited for the assessment of the impact of gene expression profile based management changes on healthcare resource utilization and patient outcome.

Furthermore, NCCN's clinical practice guideline on “Melanoma” (Version 2.2016) states that “While there is interest in newer prognostic molecular techniques such as gene expression profiling to differentiate benign from malignant neoplasms, or melanomas at low- versus high-risk for metastasis, routine (baseline) genetic testing of primary cutaneous melanomas (before or following SLNB [sentinel lymph node biopsy) is not recommended outside of a clinical study (trial)”.

**Molecular Diagnostics for Thyroid Cancer**

Molecular markers associated with thyroid cancer have been proposed to assist in determining malignancy and to guide surgery decisions for individuals with indeterminate fine needle aspiration (FNA) thyroid nodule cytopathology.

Thyroid nodules are abnormal growths or lumps that develop in the thyroid gland. While most are benign (not cancerous), a small percent are malignant (cancerous). To determine malignancy, fine needle aspiration (FNA) is used to obtain a specimen (aspirate) from the nodule which is evaluated by cytopathology and classified based on the results. Most are classified as benign
(70 to 75%) and a small percentage as malignant (5% to 10%). Approximately 25% are classified as indeterminate (unable to determine a diagnosis) and warrant further evaluation, which often includes thyroid surgery and histopathologic evaluation of thyroid tissue. However, nearly 80% of indeterminate nodules are benign based upon histopathology results.

Thyroid gene expression classifier tests and thyroid cancer mutation analysis of fine needle aspirates in thyroid nodules differ from genetic testing. Genetic testing, also known as germline mutation testing, analyzes an individual’s DNA and can identify genetic mutations to determine inherited risk of disease. An individual’s germline DNA is constant and identical in all body tissue types. RNA activity is measured by gene expression analysis. It is dynamic and responds to cellular environmental signals. Mutation analysis of fine needle aspirates or tumor tissue determines DNA mutations that have been acquired over an individual’s lifetime. These DNA changes are present only in the tissue sampled, are not usually representative of an individual’s germline DNA and are not inheritable. For information regarding gene testing (also known as germline mutation testing) for thyroid cancer (eg, multiple endocrine neoplasia [MEN]), see CPB 319 - RET Proto-oncogen Testing.

Guidelines on thyroid carcinoma from the National Comprehensive Cancer Network (NCCN, 2014) state: "Molecular diagnostic testing to detect individual mutations (e.g., BRAF, RET/PTC, RAS, PAX8/PPAR [peroxisome proliferator-activated receptors] gamma) or pattern recognition approaches using molecular classifiers may be useful in the evaluation of FNA samples that are indeterminate to assist in management decisions. The choice of the precise molecular test depends on the cytology and the clinical question being asked."

Indeterminate groups include: 1) follicular or Hurthle cell neoplasms; and 2) AUS/FLUS. The NCCN Panel recommends (category 2B) molecular diagnostic testing for evaluating FNA results that are suspicious for: 1) follicular or Hurthle cell neoplasms; or 2) AUS/FLUS (see Nodule Evaluation in the NCCN Guidelines for Thyroid Carcinoma). For the 2014 update, the NCCN Panel revised the recommendation for molecular
diagnostic testing from category 2A to category 2B for indeterminate FNA results based on a series of panel votes. The panel noted that the molecular testing (both the Gene Expression Classifier and the individual mutation analysis) was available in the majority of NCCN Member Institutions (>75%). About 70% of the panelists would recommend using a gene expression classifier in the evaluation of follicular lesions. The gene expression classifier measures the expression of at least 140 genes. BRAF mutation analysis was recommended by 50% of the panelists in the evaluation of thyroid nodules (not restricted to the follicular lesions). Furthermore, about 60% of the panelists would recommend BRAF testing in the evaluation of follicular lesions. A minority of panelists expressed concern regarding observation of follicular lesions because they were perceived as potentially premalignant lesions with a very low, but unknown, malignant potential if not surgically resected (leading to a recommendation for either observation or definitive surgical resection in lesions classified as benign by molecular testing). Rather than proceeding to immediate surgical resection to obtain a definitive diagnosis for these intermediate FNA cytology groups (follicular lesions), patients can be followed with observation if the application of a specific molecular diagnostic tests results in a predicted risk of malignancy that is comparable to the rate seen in cytologically benign thyroid FNAs (approximately < 5%). NCCN guidelines state that it is important to note that the predictive value of molecular diagnostics may be significantly influenced by the pre-test probability of disease associated with the various FNA cytology groups. Furthermore, in the cytologically indeterminate groups, the risk of malignancy for FNA can vary widely between institutions. Because the published studies have focused primarily on adult patients with thyroid nodules, the diagnostic utility of molecular diagnostics in pediatric patients remains to be defined. Therefore, proper implementation of molecular diagnostics into clinical care requires an understanding of both the performance characteristics of the specific molecular test and its clinical meaning across a range of pre-test disease probabilities.

For support for use of a gene classifier, the NCCN guidelines reference validation studies of the Afirma Thyroid FNA Analysis (Alexander et al, 2012; Chudova et al, 2010; Kloos, et al., 2013;
McIver, et al., 2014) and Thyroseq (Nikiforov, et al., 2009; Ohori, et al., 2010; Nikiforov, et al., 2011). These studies demonstrate that this molecular diagnostic meets NCCN threshold of predicting malignancy of 5 % or less (i.e., a negative predictive value of 95 %), allowing physicians to observe an indeterminate thyroid nodule in lieu of surgery.

Guidelines from the American Thyroid Association (2015) state that, "if molecular testing is being considered, patients should be counseled regarding the potential benefits and limitations of testing and about the possible uncertainties in the therapeutic and long-term clinical implications of results. This is a strong recommendation, based upon low quality evidence. The guidelines state that the largest studies of preoperative molecular markers in patients with indeterminate FNA cytology have respectively evaluated a seven-gene panel of genetic mutations and rearrangements (BRAF, RAS, RET/PTC, PAX8/PPARc), a gene expression classifier (167 GEC; mRNA expression of 167 genes), and galectin-3 immunohistochemistry (cell blocks). The guidelines note that these respective studies have been subject to various degrees of blinding of outcome assessment. The guidelines state that "there is currently no single optimal molecular test that can definitively rule in or rule out malignancy in all cases of indeterminate cytology, and long-term outcome data proving clinical utility are needed."

Guidelines from the American Association of Clinical Endocrinologists (Gharib, et al., 2016) state that molecular testing should be considered to complement not replace cytologic evaluation, where the results are expected to influence clinical management. As a general rule, not recommended in nodules with established benign or malignant cytologic characteristics. The guidelines recommend considering the detection of BRAF and RET/PTC and, possibly, PAX8/PPARG and RAS mutations if such detection is available. The guidelines state that, because of the insufficient evidence and the limited follow-up, they do not recommend either in favor of or against the use of gene expression classifiers (GECs) for cytologically indeterminate nodules.
Thyroid gene expression classifier (GEC) (e.g., Afirma Gene Expression Classifier) is a messenger ribonucleic acid (mRNA) gene expression assay that analyzes thyroid FNA specimens to classify indeterminate nodules using a proprietary algorithm. The thyroid GEC analyzes the mRNA expression of 167 genes. The thyroid GEC is described as a "rule-out" test because a negative (i.e., benign) result rules out the presence of cancer.

Thyroid malignancy classifier tests (e.g., Afirma MTC, Afirma BRAF) purport to analyze thyroid nodules that have been classified as "malignant" or "suspicious for malignancy" through cytopathology or as "suspicious for malignancy" on GEC. Afirma MTC was developed to identify the presence of medullary thyroid cancer (MTC) while Afirma BRAF was designed to determine the presence of BRAF V600E mutation. Both are automatically initiated in the lab, if ordered by a physician, after a thyroid nodule has been classified as having abnormal findings. This process is referred to as reflex testing.

Thyroid cancer targeted mutational analysis of thyroid FNA samples has also been proposed to detect individual gene mutations associated with thyroid cancer and include BRAF V600E, RAS (HRAS, KRAS, NRAS), RET/PTC, PAX8/PPARgamma, PIK3CA.

Thyroid cancer mutational panel using next generation sequencing (NGS) (e.g., ThyroSeq) analyzes deoxyribonucleic acid (DNA) and RNA in FNA thyroid samples to determine the presence of gene mutations associated with thyroid cancer.

Quest Diagnostics offers a molecular test panel designed to help physicians determine if a thyroid gland is cancerous and requires surgical removal. The test includes the seven gene panel of mutations and rearrangements addressed by the American Thyroid Association for the clinical management of indeterminate thyroid biopsies. According to Quest Diagnostics, the Quest Diagnostics Thyroid Cancer Mutation Panel aids in detecting cancer in thyroid biopsies which are found to be indeterminate for cancer by current cytology test methods. Approximately 15% to 20% of these biopsies, which are collected by fine needle
aspiration (FNA), produce indeterminate results. An unclear result may increase the risk that a physician, in an abundance of caution, will biopsy additional tissue using a larger needle or surgically remove part or all of a thyroid suspected of having cancer that is later diagnosed as healthy. About 300,000 thyroid FNA biopsy procedures are performed annually in the United States. The panel identifies mutations of the molecular markers BRAF V600E, RAS, RET/PTC, and PAX8/PPAR gamma, which are associated with papillary and follicular thyroid cancer, two common forms of the disease. The manufacturer states that practice guidelines from the American Thyroid Association recommend that physicians consider these markers as aids in clinical management of patients with indeterminate biopsy test results. Results of a Quest Diagnostics study found that 90 of 149 FNA specimens, or about 60%, had mutations of one or more of the four markers tested by the new panel (Reitz, et al., 2014). The authors of the study stated that the presence of the four markers was generally mutually exclusive, suggesting potential value in a hierarchical screening strategy for samples with limited tissue. According to the American Cancer Society, about two tests in every 10 may need to be repeated because the sample does not contain enough cells for testing.

Thyroid cancer mutational panel (eg, ThyGenX [formerly miRInform], Thyroid Cancer Mutational Panel) evaluates thyroid FNA samples to detect gene mutations associated with thyroid cancer (eg, BRAF V600E, RAS [HRAS, KRAS, NRAS], RET/PTC, PAX8/PPARgamma, PIK3CA). Mutational panels are described as "rule-in" tests because a positive result indicates that a nodule is at high risk for malignancy; therefore, identifies or, rules in, cancer. However, because these mutations occur infrequently overall in thyroid cancer, a negative result does not rule out cancer.

Thyroid microRNA (miRNA) GEC (eg, RosettaGX Reveal, ThyraMIR) measures the expression levels of microRNAs to supposedly classify thyroid nodules with indeterminate FNA cytology. miRNA GEC may be offered alone or in combination with a thyroid cancer mutational panel (ie, ThyraMIR and ThyGenX ) to purportedly enhance specificity and sensitivity testing results.
ThyraMIR thyroid miRNA classifier is a PCR-based microRNA (miRNA) gene expression classifier that examines the expression levels of 10 miRNA genes within FNA biopsy: miR-29-1-5p, miR-31-5p, miR-138-1-3p, miR-139-5p, miR-146b-5p, miR-155, miR-204-5p, miR-222-3p, miR-375, and miR-551b-3p. It is performed following a negative ThyGenX result for all mutations or when mutations detected are not fully indicative of malignancy (i.e., ThyGenX results which favor a benign nodule but cancer could still be present). The test is used on the same FNA cytology sample. The ThyraMIR test reports a qualitative positive or negative result based on the gene expression levels.

A study combining seven-gene mutational testing (ThyGenX) with expression of a set of 10 miRNA genes (ThyraMIR) on preoperative FNA sampling from 109 patients with indeterminate cytology, showed 89% sensitivity, 85% specificity, with a 73% PPV and 94% NPV on this group with a 32% prevalence of malignancy (Labourier, et al., 2015). Labourier, et al. (2015) reported that testing with ThyGenX and ThyraMIR for DNA, mRNA, and miRNA can accurately classify benign and malignant thyroid nodules, increase the diagnostic yield, and further improve the preoperative risk-based management of benign thyroid nodules with indeterminate cytology. Labourier, et al. (2015) tested surgical specimens and preoperative FNAs (n = 638) for 17 validated gene alterations using the miRInform Thyroid test (ThyGenX) and with a 10-miRNA gene expression classifier (ThyraMIR) generating positive (malignant) or negative (benign) results. Cross-sectional sampling of thyroid nodules with atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS) or follicular neoplasm/suspicious for a follicular neoplasm (FN/SFN) cytology (n = 109) was conducted at 12 endocrinology centers across the United States. Qualitative molecular results were compared with surgical histopathology to determine diagnostic performance and model clinical effect. Mutations were detected in 69% of nodules with malignant outcome. Among mutation-negative specimens, miRNA testing correctly identified 64% of malignant cases and 98% of benign cases. The diagnostic sensitivity and specificity of the combined algorithm was 89% (95% confidence interval [CI], 73-97%) and 85% (95% CI, 75-92%), respectively. At 32% cancer prevalence,
61% of the molecular results were benign with a negative predictive value of 94% (95% CI, 85-98%). Independently of variations in cancer prevalence, the test increased the yield of true benign results by 65% relative to mRNA-based gene expression classification and decreased the rate of avoidable diagnostic surgeries by 69%.

In a news article on tests for indeterminate thyroid nodules, Tucker (2015) reviewed the data from Labourier, et al. (2015) plus additional abstracts on ThyGenX/ThyraMIR presented at the American Association of Clinical Endocrinologists' 2015 Annual Scientific and Clinical Congress, The article quoted AACE immediate past president R Mack Harrell, MD, as cautioning that "more validation is needed in real-world settings with larger numbers for this new platform, as well as for other 'next-generation' molecular tests such as the ThyroSeq .... So much of the predictive value of these tests depends on what you start with. If you start with a highly selected tertiary-care-referral cancer community, the efficacy of the test is completely different from starting with a practice that's receiving every thyroid nodule in town. So, it needs to be tested in a true community-practice-type setting with lots of patients before you can be sure exactly how it's going to perform”.

Lithwick-Yanai, et al. (2016) sought to develop an assay, the RosettaGX Reveal, that could classify indeterminate thyroid nodules as benign or suspicious, using routinely prepared fine needle aspirate (FNA) cytology smears. A training set of 375 FNA smears was used to develop the microRNA-based assay, which was validated using a blinded, multicenter, retrospective cohort of 201 smears. Final diagnosis of the validation samples was determined based on corresponding surgical specimens, reviewed by the contributing institute pathologist and two independent pathologists. Validation samples were from adult patients (≥18 years) with nodule size >0.5 cm, and a final diagnosis confirmed by at least one of the two blinded, independent pathologists. The developed assay differentiates benign from malignant thyroid nodules, using quantitative RT-PCR. Test performance on the 189 samples that passed quality control: negative predictive value: 91% (95% CI 84% to 96);
sensitivity: 85% (CI 74% to 93%); specificity: 72% (CI 63% to 79%). Performance for cases in which all three reviewing pathologists were in agreement regarding the final diagnosis (n=150): negative predictive value: 99% (CI 94% to 100%); sensitivity: 98% (CI 87% to 100%); specificity: 78% (CI 69% to 85%). The authors concluded that this assay utilizing microRNA expression in cytology smears distinguishes benign from malignant thyroid nodules using a single FNA stained smear, and does not require fresh tissue or special collection and shipment conditions. The authors stated that this assay offers a valuable tool for the preoperative classification of thyroid samples with indeterminate cytology. Limitations of this study include its small size and large number of post hoc exclusions to create a set in which all three pathologists were in agreement.

Benjamin, et al. (2016) reported on the analytical validation of the RosettaGX Reveal assay. More than 800 FNA slides were tested, including slides stained with Romanowsky-type and Papanicolaou stains. The assay was examined for the following features: intranodule concordance, effect of stain type, minimal acceptable RNA amounts, performance on low numbers of thyroid cells, effect of time since sampling, and analytical sensitivity, specificity, and reproducibility. The authors reported that the assay can be run on FNA slides for which as little as 1% of the cells are thyroid epithelial cells or from which only 5 ng of RNA have been extracted. Samples composed entirely of blood failed quality control and were not classified. Stain type did not affect performance. All slides were stored at room temperature. However, the length of time between FNA sampling and processing did not affect assay performance. There was a high level of concordance between laboratories (96%), and the concordance for slides created from the same FNA pass was 93%. The authors concluded that the microRNA-based assay was robust to various physical processing conditions and to differing sample characteristics. The authors concluded that given the assay’s performance, robustness, and use of routinely prepared FNA slides, it has the potential to provide valuable aid for physicians in the diagnosis of thyroid nodules.

Bhatia et al (2015) noted that FNA cytology, being the mainstay to
diagnose thyroid nodules, does not provide definitive results in a subset of patients. The use of molecular markers testing has been described as a useful aid in differentiation of thyroid nodules that present with an indeterminate cytodiagnosis. Molecular tests, such as the Afirma gene classifier, mutational assay and immunohistochemical markers have been increasingly used to further increase the accuracy and defer unnecessary surgeries for benign thyroid nodules. However, in light of the current literature, their emerging roles in clinical practice are limited due to financial and technical limitations. Nevertheless, their synergistic implementation can predict the risk of malignancy and yield an accurate diagnosis. This review discussed the clinical utility of various molecular tests done on FNA indeterminate nodules to avoid diagnostic thyroidectomies and warrant the need of future multi-Institutional studies.

ROMA

Risk of Ovarian Malignancy Algorithm (ROMA) is a blood test cleared by the FDA to aid in the evaluation of pelvic masses for the likelihood of malignancy before surgery. ROMA measures human epididymis protein 4 (HE4) and CA-125. These measurements are applied to an algorithm, combined with menopausal status, to calculate a numerical score.

The BCBS TEC’s assessment on “Multi-Analyte Testing for the Evaluation of Adnexal Masses” (2013) concluded that ROMA does not meet TEC criteria. It noted that “evidence regarding the effect of ... ROMA and effects on health outcomes is indirect, and based on studies of diagnostic performance of the tests in patients undergoing surgery for adnexal masses. Although the studies show improvements in sensitivity and worsening of specificity with the use of the tests in conjunction with clinical assessment, there are problems in concluding that this results in improved health outcomes. The clinical assessment performed in the studies is not well characterized... ROMA does not improve the sensitivity of testing to a great extent. Underlying these issues is some uncertainty regarding the benefit of initial treatment by a gynecologic oncologist beyond the need for reoperation is some cases”.
Guidelines on ovarian cancer from the National Comprehensive Cancer Network (NCCN, 2016) state that "it has been suggested that specific biomarkers (serum HE4 and CA-125) along with an algorithm (Risk of Ovarian Malignancy Algorithm [ROMA]) may be useful for determining whether a pelvic mass is malignant or benign. The FDA has approved the use of HE4 and CA-125 for estimating the risk for ovarian cancer in women with a pelvic mass. Currently, the NCCN Panel does not recommend the use of these biomarkers for determining the status of an undiagnosed pelvic mass."

Guidelines on management of adnexal masses from the American College of Obstetricians and Gynecologists (ACOG, 2017) state that ROMA includes HE4, which has been found to be more sensitive and specific than CA 125 for the evaluation of adnexal masses. The guidelines state that serum biomarker panels [OVA1 and ROMA] may be used as an alternative to CA 125 alone in determining the need for referral to or consultation with a gynecological oncologist when an adnexal mass requires surgery. The guidelines state that trials that have evaluated the predictive value of these panels show potential for improved specificity; "[h]owever, comparative research has not yet defined the best testing approach."

The UK National Institute for Health Research Health Technology Assessment Programme has commissioned an assessment (Westwood, et al., 2016) comparing the Risk of Malignancy Index (RMI) to alternative risk scores for ovarian cancer, including the ROMA score, as well as Overa/OVA2 (Vermillion), simple rules ultrasound classification system (IOTA), Assessment of Different NEoplasias in the adnexa (ADNEX) model (IOTA group). The assessment is scheduled to be completed in 2017.

ERCC1

Yu and colleagues (2012) stated that the excision repair cross-complementation group 1 (ERCC1) plays an essential role in DNA repair and has been linked to resistance to platinum-based anticancer drugs among advanced NSCLC patients. These investigators examined if ERCC1 Asn118Asn and C8092A genetic
variants are associated with treatment response of platinum chemotherapy. They performed a meta-analysis using 10 eligible cohort studies (including 11 datasets) with a total of 1,252 NSCLC patients to summarize the existing data on the association between the ERCC1 Asn118Asn and C8092A polymorphisms and response to platinum regiments. Odds ratio or hazard ratio with 95 % CI were calculated to estimate the correlation. These researchers found that neither ERCC1 C8092A polymorphism nor Asn118Asn variant is associated with different response of platinum-based treatment among advanced NSCLC patients. Additionally, these 2 genetic variants are not related to treatment response in either Caucasian patients or Asian patients. The authors concluded that the findings of this meta-analysis indicated that the ERCC1 Asn118Asn and C8092A polymorphisms may not be good prognostic biomarkers for platinum-based chemotherapy in patients with stage III-IV NSCLC.

Wang et al (2012) performed a meta-analysis by using 20 eligible studies to examine polymorphisms of ERCC1, GSTs, TS and MTHFR in predicting clinical outcomes (response rate, OS and toxicity) of gastric cancer (GC) patients treated with platinum/5-Fu-based chemotherapy. The association was measured using random/fixed effect odds ratios (ORs) or hazard ratios (HRs) combined with their 95 % CIs according to the studies' heterogeneity. Statistical analysis was performed with the software STATA 9.0 package. No significant association was found between response rate and genetic polymorphism in TS, MTHFR, ERCC1, GSTM1 and GSTP1. However, response rate was higher in GSTT1 (+) genotype compared with GSTT1 (‐) genotype (T‐/T+: OR = 0.67, 95 % CI: 0.47 to 0.97). With regard to long-term outcomes, these researchers observed a significant longer OS in TS 3R/3R [(2R2R+2R3R)/3R3R: HR = 1.29, 95 % CI: 1.02 to 1.64] and GSTP1 GG/GA [(GG+AG)/AA: HR = 0.51, 95 % CI: 0.39 to 0.67] genotypes. In addition, significant association was demonstrated between toxicity and genetic polymorphism in TS, MTHFR and GSTP1 in included studies. The authors concluded that polymorphisms of ERCC1, GSTs, TS and MTHFR were closely associated with clinical outcomes of GC patients treated with platinum/5-Fu-based chemotherapy. Moreover, they state that studies with large sample size using the method of multi-variant
analyses may help us to give more persuasive data on the putative association in future.

In a meta-analysis, Gong and colleagues (2012) examined if RRM1 expression is associated with the clinical outcome of gemcitabine-containing regimen in advanced NSCLC. An electronic search was conducted using the databases PubMed, Medline, EMBASE, Cochrane library and CNKI, from inception to May, 2011. A systemic review of the studies on the association between RRM1 expression in advanced NSCLC and clinical outcome of gemcitabine-containing regimen was performed. Pooled odds ratios (OR) for the response rate, weighted median survival and time to progression were calculated using the software Revman 5.0. The search strategy identified 18 eligible studies (n = 1,243). Response rate to gemcitabine-containing regimen was significantly higher in patients with low/negative RRM1 (OR = 0.31, 95 % CI: 0.21 to 0.45, p < 0.00001). Non-small cell lung cancer SCLC patients with low/negative RRM1 who were treated with gemcitabine-containing regimen survived 3.94 months longer (95 % CI: 2.15 to 5.73, p < 0.0001) and had longer time to progression for 2.64 months (95 % CI: 0.39 to 4.89, p = 0.02) than those with high/positive RRM1. The authors concluded that low/negative RRM1 expression in advanced NSCLC was associated with higher response rate to gemcitabine-containing regimen and better prognosis. Moreover, they stated that large phase III randomized trials are needed to identify whether RRM1 detection is clinically valuable for predicting the prognosis and sensitivity to gemcitabine-containing regimen in advanced NSCLC.

Friboulet et al (2013) stated that the ERCC1 protein is a potential prognostic biomarker of the effectiveness of cisplatin-based chemotherapy in NSCLC. Although several ongoing trials are evaluating the level of expression of ERCC1, no consensus has been reached regarding a method for evaluation.

Besse et al (2013) noted that somatic ERCC1 and ribonucleotide reductase M1 (RRM1) expression levels have been extensively explored as markers of DNA repair capacity in tumor cells. Although low ERCC1 and/or RRM1 expression is generally associated with sensitivity to platinum, the results published in
retrospective and prospective studies are not always consistent. These researchers examined the function of these 2 biomarkers as well as the tools available for their assessment and the associated technical issues. Their prognostic and predictive values were summarized and considered in terms of customizing systemic therapy according to biomarker (ERCC1 and RRM1) expression levels. The authors discussed why the use of both markers should at this point be restricted to clinical research.

**GSTP1**

Prostate cancer is the most common cancer in men and the second leading cause of cancer-related deaths in the US. Due to the reportedly high false-negative rate of initial biopsy results after elevated PSA level, new approaches for improved detection in prostate cancer are needed. Several studies have shown that hypermethylation of the promoter regions of the GST-P1 and APC genes occurs at a significantly higher frequency in prostate cancer samples than in benign conditions of the prostate gland. Hypermethylation of the promoter regions of the GST-P1 and APC genes can aid in prognosticating for prostate cancer (Raman, et al., 2013).

Trock et al (2012) noted that hypermethylation of genes such as glutathione-S-transferase P1 (GSTP1) and adenomatous polyposis coli (APC) occurs with high frequency in prostate tumor tissue but is much less common in the benign prostate; however, the potential value of gene methylation biomarkers as an adjunct to biopsy histopathology has had little study. When measured in histologically benign prostate biopsy tissue, APC gene hypermethylation was found to have high negative-predictive value (NPV) and high sensitivity. GSTP1 hype rmethylation was found to have lower performance than APC. These investigators evaluated the performance of DNA methylation biomarkers in the setting of repeat biopsy in men with an initially negative prostate biopsy but a high index of suspicion for missed prostate cancer. They prospectively evaluated 86 men with an initial histologically negative prostate biopsy and high-risk features. All men underwent repeat 12-core ultrasonography-guided biopsy. DNA methylation of GSTP1 and APC was determined using tissue from
the initially negative biopsy and compared with histology of the repeat biopsy. The primary outcome was the relative NPV of APC compared with GSTP1, and its 95 % CI. On repeat biopsy, 21/86 (24 %) men had prostate cancer. APC and GSTP1 methylation ratios below the threshold (predicting no cancer) produced a NPV of 0.96 and 0.80, respectively. The relative NPV was 1.2 (95 % CI: 1.06 to 1.36), indicating APC has significantly higher NPV. Methylation ratios above the threshold yielded a sensitivity of 0.95 for APC and 0.43 for GSTP1. Combining both methylation markers produced a performance similar to that of APC alone. APC methylation patterns were consistent with a possible field effect or occurrence early in carcinogenesis. The authors concluded that APC methylation provided a very high NPV with a low percentage of false-negatives, in the first prospective study to evaluate performance of DNA methylation markers in a clinical cohort of men undergoing repeat biopsy. They stated that the potential of APC methylation to reduce unnecessary repeat biopsies warrants validation in a larger prospective cohort.

In a systematic review and meta-analysis, Yu and colleagues (2013) examined the association between GSTP1 Ile105Val polymorphism and prostate cancer (PCa) in different inheritance models. A total of 13 eligible studies were pooled into this meta-analysis. There was significant association between the GSTP1 Ile158Val variant genotypes and PCa for Ile/Ile versus Val/Val comparison [odds ratio (OR) = 0.705; I 2 = 63.7 %; 95 % CI: 0.508 to 0.977], Ile/Val versus Val/Val comparison (OR = 0.736; I 2 = 8.0 %; 95 % CI: 0.613 to 0.883), and dominant model (OR = 0.712; I 2 = 45.5 %; 95 % CI: 0.555 to 0.913). However, no associations were detected for other genetic models. In the stratified analysis by ethnicity, significant associations between GSTP1 Ile105Val polymorphism and PCa risk were also found among Caucasians (Ile/Ile versus Val/Val comparison OR = 0.818, I 2 = 0.0 %, 95 % CI: 0.681 to 0.982; Ile/Val versus Val/Val comparison OR = 0.779, I 2 = 0.0 %, 95 % CI: 0.651 to 0.933; and dominant model OR = 0.794, I 2 = 0.0 %, 95 % CI: 0.670 to 0.941), while there were no associations found for other genetic models. However, no associations were found in Asians and African-Americans for all genetic models when stratified by ethnicity. The authors concluded that the findings of this meta-analysis indicated that
GSTP1 Ile105Val polymorphisms contributed to the PCa susceptibility. However, they stated that a study with the larger sample size is needed to further evaluate gene-environment interaction on GSTP1 Ile105Val polymorphisms and PCa risk.

An assessment by the Swedish Office of Health Technology Assessment (SBU, 2011) concluded that the scientific evidence is insufficient to determine the diagnostic accuracy of the me-GSTP1 urine test.

CEACAM6

An UpToDate review on "Screening for breast cancer" (Fletcher, 2013) does NOT mention the use of carcinoembryonic antigen cell adhesion molecule 6 (CEACAM6). Also, the NCCN's clinical practice guideline on "Breast Cancer" (Version 2.2013) does NOT mention the use of carcinoembryonic antigen cell adhesion molecule 6 (CEACAM6).

DCIS Recurrence Score

An UpToDate review on “Ductal carcinoma in situ: Treatment and prognosis” (Collins et al, 2013) states that “A DCIS Recurrence Score utilizing a multigene assay has been developed and a prospective evaluation of this assay was performed using tumors from 327 patients who participated in the aforementioned E5194 trial. In a preliminary analysis, patients were stratified by recurrence score into three groups that were associated the following risks of an ipsilateral breast event (DCIS or invasive breast cancer) or invasive breast cancer:

- Low (less than 39) -- 12 and 5 %, respectively
- Intermediate (39 to 54) -- 25 and 9 %, respectively
- High (greater than or equal to 55) -- 27 and 19 %, respectively

These results suggest that the DCIS score may help select patients who should undergo adjuvant radiation. However, further validation of these results is required before the multigene assay can become a part of clinical practice. It is also worth noting that a 12 percent risk of an ipsilateral breast event at 10 years in the
lowest risk category may not be low enough to justify the routine omission of post-excision RT”.

MyPRS

MyPRS Plus (Signal Genetics) analyzes all of the nearly 25,000 genes in a patient’s genome to determine the gene expression profile that is associated with their condition (Raman, et al., 2013). In the case of myeloma, the gene expression profile is made up of the 70 most relevant genes which aid in the prediction of the patient’s outcome. MyPRS helps patients and physicians determine the best treatment for patients with Myeloma.

The NCCN’s clinical practice guideline on multiple myeloma (MM) (Version 2.2013) stated that “Further understanding of the molecular subtypes of MM is emerging from the application of high-throughput genomic tools such as gene expression profiling (GEP). With the currently available novel treatment approaches, a majority of patients with MM can now anticipate long-term disease control. However, patients with cytogenetically and molecularly defined high-risk disease do not receive the same benefit from current approaches as low-risk patients. GEP is a powerful and fast tool with the potential to provide additional prognostic value to further define risk-stratification, help therapeutic decisions, and inform novel drug design and development. At the present time, standardized testing for GEP is not available and there is inadequate data to determine how this prognostic information should be used to direct patient management”. The NCCN guideline does not include a specific recommendation for the use of the MyPRS test in risk-stratification or determining prognosis in the clinical management of patients with MM.

Oxnard et al (2013) stated that the identification of oncogenic driver mutations underlying sensitivity to EGFR and anaplastic lymphoma kinase tyrosine kinase inhibitors has led to a surge of interest in identifying additional targetable oncogenes in NSCLC. A number of new potentially oncogenic gene alterations have been characterized in recent years, including BRAF mutations,
HER2 insertions, phosphatidylinositol-4,5-bisphosphonate 3-kinase, catalytic subunit alpha polypeptide gene (PIK3CA) mutations, fibroblast growth factor receptor 1 (FGFR1) amplifications, discoidin domain receptor 2 (DDR2) mutations, ROS1 re-arrangements, and RET re-arrangements. These investigators discussed the techniques used to discover each of these candidate oncogenes, the prevalence of each in NSCLC, the pre-clinical data supporting their role in lung cancer, and data on small molecular inhibitors in development.

**NRAS**

Janku et al (2013) noted that despite development of new therapies, metastatic colorectal cancer (mCRC) largely remains an incurable disease. Approximately 2 to 6% of colorectal cancers harbor NRAS mutations. The anti-VEGF antibody bevacizumab is a backbone of most therapeutic regimens; however, biomarkers predicting its activity are not known. These investigators reported 2 cases of mCRC with a Q61K NRAS mutation that had a favorable response to bevacizumab and the histone deacetylase inhibitor valproic acid. In contrast, none of 10 patients with wild-type NRAS or unknown NRAS status and mutated KRAS (NRAS and KRAS mutations are mutually exclusive) responded to the same regimen. The authors concluded that these results suggested that NRAS mutation merits further investigation as a potential biomarker predicting the efficacy of bevacizumab-based treatment.

The EGAPP EWG (2013) found insufficient evidence to recommend for or against testing for mutations in NRAS, and/or loss of expression of PTEN or AKT proteins. The level of certainty for this evidence was low. In the absence of supporting evidence, and with consideration of other contextual issues, the EWG discourages the use of these tests in guiding decisions on initiating anti-EGFR therapy with cetuximab or panitumumab unless further evidence supports improved clinical outcomes.

*Phosphatidylinositol-4,5-bisphosphonate 3-kinase, catalytic subunit alpha polypeptide gene (PIK3CA)*
The EGAPP EWG (2013) found insufficient evidence to recommend for or against testing for mutations in \textit{PIK3CA}. The level of certainty for this evidence was low. In the absence of supporting evidence, and with consideration of other contextual issues, the EWG discourages the use of these tests in guiding decisions on initiating anti-EGFR therapy with cetuximab or panitumumab unless further evidence supports improved clinical outcomes.

Guidelines from the American Society for Clinical Oncology (Sepulveda, et al., 2017) stated: "There is insufficient evidence to recommend PIK3CA mutational analysis of colorectal carcinoma tissue for therapy selection outside of a clinical trial (Type: no recommendation; Strength of Evidence: insufficient, benefits/harms balance unknown; Quality of Evidence: insufficient)."

\textit{Cyclin D1 and FADD}

Cyclin D1 is used to diagnose of mantle cell lymphoma and predict recurrence of disease (Chin, et al., 2006). D-type cyclins are predominantly expressed in the G1 phase of the cell cycle. The expression pattern of cyclin D1 has been extensively studied in certain cancer types including lymphoma and non-small cell lung cancer. Approximately 30 percent of breast carcinomas are Cyclin D1 positive. Over expression of Cyclin D1 is now a well established criterion for the diagnosis of Mantle Cell Lymphoma, a malignant, non-Hodgkin's lymphoma which is characterized by a unique chromosomal translocation t(11;14).

Rasamny et al (2012) stated that cyclin D1 and FADD (Fas-associated protein with death domain) regulate the cell cycle and apoptosis, respectively, and are located on chromosome 11q13, which is frequently amplified in head and neck squamous cell carcinoma (HNSCC). This study evaluated these proteins as predictors of clinical outcomes for HNSCC. A total of 222 patients with upper aero-digestive HNSCC were included in this study. Patients with tumors that were strongly positive for cyclin D1 and FADD had reduced OS (p = 0.003 and p < 0.001), disease-specific survival (DSS; p = 0.039 and p < 0.001), and DFS (p = 0.026 and p <
0.001) survival, respectively. Together, the 2 markers effectively stratified OS (p < 0.001), DSS (p < 0.001), and DFS (p = 0.002).
Strong FADD staining correlated with greater alcohol consumption and varied significantly with primary tumor site: 56% of hypopharynx tumors expressed high levels of FADD but only 7% of glottis tumors. Using Cox regression analysis, FADD and N stage were significant independent predictors of DSS and DFS, whereas cyclin D1, FADD, and N stage were independently significant for OS. The authors concluded that cyclin D1 and FADD may have utility as predictors of long-term outcomes for patients with HNSCC. Moreover, they stated that further study is needed to determine if these proteins predict response to different treatment approaches or assist in selecting patients for multi-modality therapy.

**Prolaris**

Prolaris (Myriad Genetics, Salt Lake City, UT) uses archived tumor specimens as the mRNA source, reverse transcriptase polymerase chain reaction amplification, and a low density RTPCR array platform. Prolaris is used to quantify expression levels of 31 cell cycle progression (CCP) genes and 15 housekeeper genes to generate a CCP score. An assessment by the BlueCross BlueShield Association Technology Evaluation Center (TEC) concluded that direct evidence is insufficient to establish the analytic validity, clinical validity, or clinical utility of the Prolaris test. The BlueCross BlueShield Association assessment (BCBSA, 2015) stated: "Published evidence is sparse and insufficient to draw conclusions on the analytic validity, clinical validity, or clinical utility of Prolaris ... in patients under active surveillance program."

An assessment by the Adelaide Health Technology Assessment (Ellery, et al., 2014) found that there is currently uncertainty around the clinical utility of Prolaris. Citing a study by Shore, et al. (2014) showing that only a small percent of urologist would definitely change treatment based on the test results, "it would appear that there is hesitancy about the use of the technology in clinical practice, and it appears that changes to clinical management based on the prognostic information provided by these genetic tests are unlikely to occur. Therefore HealthPACT
recommends that no further research be conducted on their behalf at this point in time."

NCCN prostate cancer guidelines (2015) state: "The Prolaris assay produces a cell cycle progression (CCP) score from RNA expression levels of 31 genes involved in CCP. . . . For example, Prolaris has been successful in 93% of radical prostatectomy specimens, and 70% of diagnostic prostate biopsy specimens. The Prolaris CCP score has been demonstrated predictive when applied in prospective-retrospective designs for biochemical recurrence or metastasis after radical prostatectomy, for survival when men were observed after diagnosis on transurethral resection of prostate or diagnostic needle biopsy, and for biochemical recurrence and survival after external beam radiation therapy. .. Prolaris has changed treatment recommendations in 32% to 65% of cases and may enhance adherence to the treatment recommended. . . Both [Prolaris and Oncotype DX Prostate] molecular biomarker tests have been developed with extensive industry support, guidance, and involvement, and have been marketed under the less rigorous FDA regulatory pathway for biomarkers. Their clinical utility awaits evaluation by prospective, randomized clinical trials, which are unlikely to be done. The marketplace and comparative effectiveness research may be the only means for these tests and others like them to gain their proper place for better risk stratification for men with clinically localized prostate cancer."

A guideline from the American Society for Clinical Oncology on active surveillance of prostate cancer (Chen, et al., 2016) stated that "[u]se of ancillary tests beyond DRE, PSA, and biopsy to improve patient selection or as part of monitoring in an AS regimen remains investigational. Although there is a potential for genomic tests that use biopsy tissue to predict patients who are more rather than less likely to have disease progression and cancer-specific mortality and for multiparametric magnetic resonance imaging (mpMRI) to guide biopsies to find more clinically aggressive disease, prospective validation of these tests is needed to assess their impact on patient outcomes such as survival. Selective use of these ancillary tests in patients with discordant clinical and/or pathologic findings may be
appropriate."

An assessment by the National Institute for Health and Care Excellence (NICE, 2016) noted that most of the relevant evidence for Prolaris is on clinical validity, and evidence for the prognostic value of Prolaris is based only on the retrospective analyses of archived material. No studies examined the prospective use of Prolaris on patient outcomes.

Guidelines on localized prostate cancer from the American Urologic Association (Sanda, et al., 2017) state based upon expert opinion that, among most low-risk localized prostate cancer patients, Prolaris and other tissue based genomic biomarkers have not shown a clear role in the selection of candidates for active surveillance. The guidelines also indicate that tissue based genomic biomarkers are not necessary for followup. The guidelines state that the Prolaris test has not been validated as providing substantial benefit in the active surveillance population.

**Oncotype Dx Prostate**

The Oncotype DX test for prostate cancer (Genomic Health) is a genomic test that determines the risk of the cancer before treatment begins (Raman, et al., 2013). The test predicts how likely it is that the cancer is low risk and contained within the prostate, or higher risk and more likely to grow and spread. With this information, the patient and their doctor can choose the most appropriate treatment option. For example, a lower risk prostate cancer with more favorable pathology, one that may not need invasive treatment and can be safely managed through close and careful monitoring – a treatment approach called active surveillance. This genomic test measures biology through the expression of 17 genes across multiple key biological pathways in prostate cancer which can predict the aggressiveness of prostate cancer providing an individualized risk assessment.

Oncotype Dx Prostate Cancer Assay (Genomic Health, Redwood City, CA) is used to quantify expression levels of 12 cancer-related and 5 reference genes to generate a Genomic Prostate Score (GPS). In the final analysis, the cell cycle progression (CCP) score
(median 1.03, interquartile range 0.41 to 1.74) and GPS (range of 0 to 100) are combined in proprietary algorithms with clinical risk criteria (PSA, Gleason grade, tumor stage) to generate new risk categories (i.e., reclassification) intended to reflect biological indolence or aggressiveness of individual lesions, and thus inform management decisions.

Klein, et al. (2014) sought to identify and validate a biopsy-based gene expression signature that predicts clinical recurrence, prostate cancer (PCa) death, and adverse pathology. Gene expression was quantified by reverse transcription-polymerase chain reaction for three studies—a discovery prostatectomy study (n=441), a biopsy study (n=167), and a prospectively designed, independent clinical validation study (n=395)—testing retrospectively collected needle biopsies from contemporary (1997-2011) patients with low to intermediate clinical risk who were candidates for active surveillance (AS). The main outcome measures defining aggressive PCa were clinical recurrence, PCa death, and adverse pathology at prostatectomy. Cox proportional hazards regression models were used to evaluate the association between gene expression and time to event end points. Results from the prostatectomy and biopsy studies were used to develop and lock a multigene-expression-based signature, called the Genomic Prostate Score (GPS); in the validation study, logistic regression was used to test the association between the GPS and pathologic stage and grade at prostatectomy. Decision-curve analysis and risk profiles were used together with clinical and pathologic characteristics to evaluate clinical utility. Of the 732 candidate genes analyzed, 288 (39%) were found to predict clinical recurrence despite heterogeneity and multifocality, and 198 (27%) were predictive of aggressive disease after adjustment for prostate-specific antigen, Gleason score, and clinical stage. Further analysis identified 17 genes representing multiple biological pathways that were combined into the GPS algorithm. In the validation study, GPS predicted high-grade (odds ratio [OR] per 20 GPS units: 2.3; 95% confidence interval [CI], 1.5-3.7; p<0.001) and high-stage (OR per 20 GPS units: 1.9; 95% CI, 1.3-3.0; p=0.003) at surgical pathology. GPS predicted high-grade and/or high-stage disease after controlling for established clinical factors (p<0.005) such as an OR of 2.1 (95% CI, 1.4-3.2) when
adjusting for Cancer of the Prostate Risk Assessment score. A limitation of the validation study was the inclusion of men with low-volume intermediate-risk PCa (Gleason score 3+4), for whom some providers would not consider AS.

Cullen, et al. (2015) used a racially diverse cohort of men (20% African American [AA]) to evaluate the association of the clinically validated 17-gene Genomic Prostate Score (GPS) with recurrence after radical prostatectomy and adverse pathology (AP) at surgery. Biopsies from 431 men treated for National Comprehensive Cancer Network (NCCN) very low-, low-, or intermediate-risk PCa between 1990 and 2011 at two US military medical centers were tested to validate the association between GPS and biochemical recurrence (BCR) and to confirm the association with AP. Metastatic recurrence (MR) was also evaluated. Cox proportional hazards models were used for BCR and MR, and logistic regression was used for AP. Central pathology review was performed by one uropathologist. AP was defined as primary Gleason pattern 4 or any pattern 5 and/or pT3 disease. GPS results (scale: 0-100) were obtained in 402 cases (93%); 62 men (15%) experienced BCR, 5 developed metastases, and 163 had AP. Median follow-up was 5.2 yr. GPS predicted time to BCR in univariable analysis (hazard ratio per 20 GPS units [HR/20 units]: 2.9; p<0.001) and after adjusting for NCCN risk group (HR/20 units: 2.7; p<0.001). GPS also predicted time to metastases (HR/20 units: 3.8; p=0.032), although the event rate was low (n=5). GPS was strongly associated with AP (odds ratio per 20 GPS units: 3.3; p<0.001), adjusted for NCCN risk group. In AA and Caucasian men, the median GPS was 30.3 for both, the distributions of GPS results were similar, and GPS was similarly predictive of outcome.

Dall'Era, et al. (2015) performed a retrospective chart review to assess the impact of incorporating the Oncotype DX Genomic Prostate Score on treatment recommendations and decisions for men with newly diagnosed low risk prostate cancer in community urology practices. A total of 24 urologists who ordered the Oncotype DX prostate cancer assay soon after launch (May 2013) were invited to participate in the study. Clinicopathological data, Genomic Prostate Score results and treatment related
information were retrieved from medical records. Data also were collected for a pre-Genomic Prostate Score baseline group diagnosed from May 2012 to April 2013. Descriptive analyses were performed to evaluate the proportion of men for whom active surveillance was recommended and used before and after the availability of Genomic Prostate Score. Overall 15 physicians contributing 211 patients (Genomic Prostate Score group 124, baseline group 87) participated in the chart review. Patients in the Genomic Prostate Score and baseline groups had comparable risk based on traditional clinical pathological features, with 82% with NCCN® very low or low risk disease. With Genomic Prostate Score the relative increase in active surveillance recommended was 22% (baseline 50% and Genomic Prostate Score 61%, absolute increase of 11%) and the relative increase in use of active surveillance was 56% (baseline 43% and Genomic Prostate Score 67%, absolute increase of 24%). Treatment recommendations for active surveillance were directionally consistent with assay reported risk.

Badani, et al. (2015) performed a prospective study to assess the impact of incorporating Oncotype Dx GPS into treatment recommendations in 3 high volume urology practices. Men with newly diagnosed prostate cancer meeting specific NCCN criteria were prospectively enrolled in the trial. Biopsy tissue was analyzed. Urologists indicated treatment recommendations on questionnaires administered before and after GPS. The primary study objectives were to assess all changes in treatment modality and/or treatment intensity after GPS. A total of 158 men were included in analysis, including 35, 71 and 52 at NCCN very low, low and low-intermediate risk. Biological risk predicted by GPS differed from NCCN clinical risk alone in 61 men (39%). Overall 18% of recommendations between active surveillance and immediate treatment changed after GPS. The relative increase in recommendations for active surveillance was 24% (absolute change 41% to 51%). In 41 of 158 men (26%) modality and/or intensity recommendations changed after GPS, including 25, 14 and 2 in whom recommendation intensity decreased, increased and were equivocal, respectively. All changes were directionally consistent with GPS. The NCCN low risk group showed the greatest absolute recommendation change after GPS (37%). In 17
of 57 men (30%) the initial recommendation of radical prostatectomy was changed to active surveillance after GPS. Urologists indicated greater confidence and found that incorporating GPS was useful in 85% and 79% of cases, respectively, including when biological risk confirmed the clinical risk category.

Brand, et al. (2016) performed a patient-specific meta-analysis (MA) of two independent clinical validation studies of a 17-gene biopsy-based genomic assay (Oncotype Dx Prostate) as a predictor of favorable pathology at radical prostatectomy. Patient-specific MA was performed on data from 2 studies (732 patients) using the Genomic Prostate Score (GPS; scale 0-100) together with Cancer of the Prostate Risk Assessment (CAPRA) score or National Comprehensive Cancer Network (NCCN) risk group as predictors of the likelihood of favorable pathology (LFP). Risk profile curves associating GPS with LFP by CAPRA score and NCCN risk group were generated. Decision curves and receiver operating characteristic curves were calculated using patient-specific MA risk estimates. Patient-specific MA-generated risk profiles ensure more precise estimates of LFP with narrower confidence intervals than either study alone. The investigators stated that GPS added significant predictive value to each clinical classifier. A model utilizing GPS and CAPRA provided the most risk discrimination. In decision-curve analysis, greater net benefit was shown when combining GPS with each clinical classifier compared with the classifier alone. The area under the receiver operating characteristic curve improved from 0.68 to 0.73 by adding GPS to CAPRA, and 0.64 to 0.70 by adding GPS to NCCN risk group. The proportion of patients with LFP >80% increased from 11% using NCCN risk group alone to 23% using GPS with NCCN. Using GPS with CAPRA identified the highest proportion-31%-of patients with LFP > 80%.

In a prospective study, Albala, et al. (2016) evaluated the clinical utility and economic impact of the Oncotype DX Prostate GPS in patients with low-risk prostate cancer. The study was conducted at a single large urology group practice and enrolled patients with a single insurance carrier. The insurance carrier calculated cost data from the first 180 days after diagnosis (including the cost of
the diagnostic biopsy) and provided the average treatment cost per patient from their analysis, and compared management patterns and costs from a baseline, untested population to a similar prospective, GPS-tested population. The primary endpoint of the study was the net percentage difference in prospective treatment decisions with use of Oncotype DX Prostate GPS as compared with the baseline treatment patterns without use of GPS. Of the 71 men in the baseline group who were NCCN very low risk and low risk, 27 (38%) were managed with AS, 25 (35%) had RP, 18 (25%) were managed with IMRT and 1 (1%) had whole-gland cryoablation. In the 51 GPS-tested NCCN very-low-risk and low-risk patients, 30 (59%) were managed with AS, 13 (25%) had an RP, 6 (12%) were managed with IMRT, 1 (2%) was managed with multimodal therapy (IMRT and brachytherapy), and 1 (2%) chose focal cryoablation. AS utilization was 21% higher in the prospective GPS-tested cohort of very-low-risk and low-risk men compared with the baseline cohort of risk-group-matched men. The rate of RP was 10% lower and the rate of IMRT was 14% lower in the prospective cohort of very-low-risk and low-risk men when GPS was incorporated into treatment decisions compared with the baseline cohort of risk group-matched men. In the 29 GPS untested NCCN intermediate-risk patients, 5 (17.2%) were managed by AS, 12 (41.4%) by RP, 11 (37.9%) by IMRT, and 1 (3.4%) by CyberKnife radiosurgery. In the 29 GPS-tested NCCN favorable intermediate-risk patients, no patient chose AS, 14 (48%) chose RP, 11 (38%) chose RT, 1 (3%) chose brachytherapy, and 3 (10%) chose multimodal treatment. AS utilization decreased and RP slightly increased in NCCN favorable intermediate-risk group patients after using GPS. IMRT usage remained unchanged between the baseline and prospective groups. Comparing payer costs in the first 180 days after diagnosis for the entire NCCN risk population (n = 80), there was an average cost addition of $1023 per patient, including the cost of the GPS at $4520 (total net addition of $81,855 for the entire GPS-tested population). Comparing payer costs in the first 180 days after diagnosis for the baseline and prospective NCCN very-low-risk and low-risk populations, there was an average savings per patient of $2286, including the cost of the GPS.

An assessment by the BlueCross BlueShield Technology Evaluation
Center (TEC, 2014) concluded that direct evidence is insufficient to establish the analytic validity, clinical validity, or clinical utility of the Oncotype Dx Prostate.

The BlueCross BlueShield Technology Evaluation Center’s assessment on “Gene Expression Analysis for Prostate Cancer Management” (BCBSA, 2015) concluded that “Evidence is insufficient to determine whether . . . Oncotype Dx Prostate testing improves health outcomes in the investigational setting. Based on the above, neither the Prolaris nor Oncotype Dx Prostate array-based gene expression test meets the TEC criteria”. The assessment stated: Published evidence is sparse and insufficient to draw conclusions on the . . . clinical validity or utility of Oncotype Dx Prostate in patients under active surveillance program.”

An assessment by Adelaide Health Technology Assessment (Ellery, et al., 2014) concluded that "there is uncertainty about the clinical utility" of the Oncotype Dx Prostate and the Prolaris tests, "even when taking into account the highest level of evidence available" The assessment stated that it remains to be verified whether genetic expression of the unique gene panels involved are robust to heterogeneous sampling of prostate tissue at the time of biopsy. Also, the need for tissue which has previously been fixed for histological analysis is of some concern. The assessment observed that this is the most obvious reason for the relatively high number of patients for whom a valid test results could not be obtained.

European Association of Urology (2015) prostate cancer guidelines state that genomics on the tissue sampling appear "promising," but "further study data will be needed before such markers can be used in standard clinical practice."

NCCN guidelines on prostate cancer (NCCN, 2016) state: "These molecular tests listed [Decipher, Ki-67, Oncotype Dx Prostate, Prolaris, ProMark, PTEN] have been developed with extensive industry support, guidance, involvement and have been marketed under the less rigorous FDA regulatory pathway for biomarkers. Although full assessment of their clinical utility requires
prospective, randomized clinical trials, which are unlikely to be done, the panel believes that men with clinically localized disease may consider the use of tumor-based molecular assays at this time. Future comparative effectiveness research may allow these tests and others like them to gain additional evidence regarding their utility for better risk stratification of men with prostate cancer.”

A guideline from the American Society for Clinical Oncology on active surveillance of prostate cancer (Chen, et al., 2016) stated that "[u]se of ancillary tests beyond DRE, PSA, and biopsy to improve patient selection or as part of monitoring in an AS regimen remains investigational. Although there is a potential for genomic tests that use biopsy tissue to predict patients who are more rather than less likely to have disease progression and cancer-specific mortality and for multiparametric magnetic resonance imaging (mpMRI) to guide biopsies to find more clinically aggressive disease, prospective validation of these tests is needed to assess their impact on patient outcomes such as survival. Selective use of these ancillary tests in patients with discordant clinical and/or pathologic findings may be appropriate."

Guidelines on localized prostate cancer from the American Urologic Association (Sanda, et al., 2017) state based upon expert opinion that, among most low-risk localized prostate cancer patients, the Oncotype Dx Prostate and other tissue based genomic biomarkers have not shown a clear role in the selection of candidates for active surveillance. The guidelines also indicate that tissue based genomic biomarkers are not necessary for followup. The guidelines state that the Oncotype Dx Prostate test has not been validated as providing substantial benefit in the active surveillance population.

Prostavysion

ProstaVysion (Bostwick Labs) is a prognostic genetic panel for prostate cancer (Raman, et al., 2013). This test examines two major mechanisms of prostate carcinogenesis: ERG gene fusion/translocation and the loss of the PTEN tumor suppressor
gene. This test is a tissue-based panel. By examining these two markers, ProstaVysion is able to provide a molecular analysis of prostate cancer aggressiveness and long-term patient prognosis.

ERG gene fusions are found in 40% of primary prostate cancers and are associated with a more aggressive phenotype. Deletion of PTEN occurs in both localized prostate cancers and 60% of metastases.

**PAM50 and Prosigna**

PAM50 Breast Cancer Intrinsic Classifier (University of Utah) examines 50 genes and sorts breast cancer into four subtypes (Raman, et al., 2013). Each subtype responds differently to standard therapies, and knowing the subtype allows doctors to tailor treatment for each patient. PAM50 assay can aid profiling for both prognosis and prediction of benefit from adjuvant tamoxifen and has been found superior to immunohistochemistry.

A National Institute for Health Research assessment (Ward, et al., 2013) found the evidence for PAM50 to be limited. The report concluded that "the evidence base for PAM50 is still relatively immature."

An international working group (Azim, et al, 2013) found insufficient evidence of the analytic and clinical validity of the PAM50. They found insufficient evidence of the clinical utility of the PAM50 or the other breast cancer genomic tests that they assessed.

A report by the Belgian Healthcare Knowledge Centre (KCE) (San Miguel, et al., 2015) found that the evidence for PAM50 is limited to studies supporting the prognostic ability (clinical validity) of the test. Most of the evidence is in node-positive patients. The KCE found insufficient evidence on the impact of PAM50 on clinical management (clinical utility).

Prosigna is intended for use as a prognostic indicator in conjunction with other clinicopathologic factors for distant recurrence-free survival at 10 years in postmenopausal women...
with hormone receptor (HR)–positive, lymph node–
negative/stage I or II, or lymph node–positive (1-3 positive
nodes)/stage II breast cancer to be treated with adjuvant
eンドロビュングtherapy alone. The assay measures the expression
profiles of genes included in the PAM50 gene signature, as well as
8 housekeeping genes (for normalization), 6 positive controls and
8 negative controls.

The BlueCross BlueShield Association (2015) concluded that the
use of Prosigna to determine recurrence risk in women with
early-stage breast cancer does not meet the TEC criteria. The
evidence is insufficient to permit conclusions regarding health
outcomes. Assay performance characteristics of the commercially
available version of the test indicate high reproducibility.

A medical technology innovation briefing by the National Institute
for Health and Clinical Excellence (NICE, 2015) noted that none of
the women analyzed in the clinical validation studies
(citing Gnant, et al. (2014), Sestak, et al. (2014) and Dowsett et al.
(2013)) had chemotherapy as part of their initial treatment. As a
result, the prognostic value of the Prosigna ROR score in a
chemotherapy-treated population is unknown. Sestak. The
briefing also noted that the populations included in the patient
cohorts included in these clinical validation studies. Sestak, et al.
(2014) combined data previously analysed by Dowsett et al.
(2013) and Gnant et al. (2014). Dowsett et al. (2013) and Sestak
et al. (2014) used the clinical treatment score as a comparator
rather than the online tools Adjuvant! Online and PREDICT, or the
NPI, which are standard practice in the UK. Similarly, Gnant et al.
(2014) used a combination score of clinicopathologic parameters
as the comparator for Prosigna. NICE stated that such indices are
always incomplete because they may not include all parameters
used by clinicians in other health systems to aid clinical decision-
making. The NICE briefing also pointed out that all included
studies received financial support or disclosed competing
interests from the manufacturer, and this introduces the potential
for bias in the reporting of outcomes.

Guidelines from the American Society for Clinical Oncology (2016)
state: "If a patient has ER/PgR-positive, HER2-negative (node-
negative) breast cancer, the clinician may use the PAM50 risk of recurrence (ROR) score (Prosigna Breast Cancer Prognostic Gene Signature Assay; NanoString Technologies, Seattle, WA), in conjunction with other clinicopathologic variables, to guide decisions on adjuvant systemic therapy." This is a strong recommendation based upon high quality evidence. The guidelines recommend against the use of PAM50 to guide decisions on adjuvant systemic therapy in patients with ER/PgR-positive, HER2-negative (node-positive) breast cancer. The guidelines also recommend against the use of PAM50 in HER2-positive breast cancer and TN breast cancer. The guidelines recommended against the use of PAM50 to guide decisions on extended endocrine therapy for patients with ER/PgR-positive, HER-2 negative (node-negative) breast cancer who have had 5 years of endocrine therapy without evidence of recurrence.

Cancer Care Ontario Guidelines (Chang, et al., 2016) state: "Although no assay represents a gold standard, Oncotype DX is supported by the widest range of evidence for prognosis and prediction of chemotherapy benefit, while both Prosigna and EndoPredict have evidence-based validity in providing some of the same or similar clinical information."

*OncotypeDx Colon*

The Oncotype Dx Colon has been promoted for use in colorectal cancer. However, there is a lack of evidence establishing the clinical utility of this test in colorectal cancer.

The results of the Quick and Simple and Reliable Study (QUASAR) were published by Gray et al (2011). The purpose of the QUASAR study was develop quantitative gene expression assays to assess recurrence risk and benefits from chemotherapy in patients with stage II colon cancer. Recurrence score (RS) and treatment score (TS) were calculated from gene expression levels of 13 cancer-related genes and from five reference genes. The results of the study showed risk of recurrence to be significantly associated with RS (95% confidence interval [CI]: 1.11 to 1.74; p = 0.004). Recurrence risks at 3 years were 12%, 18%, and 22% for
predefined low, intermediate, and high recurrence risk groups, respectively. Continuous RS was associated with risk of recurrence (p = 0.006), but there was no trend for increased benefit from chemotherapy at higher TS (p = 0.95). The continuous 12-gene RS has been validated in a prospective study for assessment of recurrence risk in patients with stage II colon cancer after surgery. RS was also found to provide prognostic value that complements T stage and mismatch repair.

Yamanaka, et al. (2016) evaluated the 12-gene Recurrence Score assay for stage II and III colon cancer without chemotherapy to reveal the natural course of recurrence risk in stage III disease. A cohort-sampling design was used. From 1,487 consecutive patients with stage II to III disease who had surgery alone, 630 patients were sampled for inclusion with a 1:2 ratio of recurrence and nonrecurrence. Sampling was stratified by stage (II v III). The assay was performed on formalin-fixed, paraffin-embedded primary cancer tissue. Association of the Recurrence Score result with recurrence-free interval (RFI) was assessed by using weighted Cox proportional hazards regression. Overall, 597 of 630 patients were analyzable-247 patients had stage II, and 350 had stage III colon cancer. The continuous Recurrence Score was significantly associated with RFI after adjustment for disease stage (hazard ratio for a 25-unit increase in Recurrence Score, 2.05; 95% CI, 1.47 to 2.86; P < .001). With respect to prespecified subgroups, as defined by low (< 30), intermediate (30 to 40), and high (≥ 41) Recurrence Score risk groups, patients with stage II disease in the high-risk group had a 5-year risk of recurrence similar to patients with stage IIIA to IIIB disease in the low-risk group (19% v 20%), whereas patients with stage IIIA to IIIB disease in the high-risk group had a recurrence risk similar to that of patients with stage IIIC disease in the low-risk group (approximately 38%).

The authors concluded that this study provides the first validation of the 12-gene Recurrence Score assay in stage III colon cancer without chemotherapy and showed the heterogeneity of recurrence risks in stage III as well as in stage II colon cancer.

The NCCN's clinical practice guideline on “Colon cancer” (Version
2.2015) states that there are insufficient data to recommend the use of multi-gene assays (e.g., the Oncotype DX colon cancer assay) to determine adjuvant therapy.

An assessment prepared for the Agency for Healthcare Research and Quality (Meleth,, 2014) stated: "For CRC, evidence did not adequately support added prognostic value for Oncotype DX Colon. evidence either did not support added prognostic value or we found no studies with sufficiently low RoB to support a conclusion about prognostic value."

The Institut national d’excellence en santé et services sociaux (INESSS) (Boily, et al., 2016) reviewed the data on Oncotype Dx Colon, noting that the Oncotype Dx Colon has prognostic value in stage II colon cancer, but is not currently reimbursed in Quebec.

Decipher

The Decipher test appears to be a RNA biomarkers “assay” for prostate cancer. Decipher does this by measuring the expression levels of 22 RNA biomarkers involved in multiple biological pathways across the genome that are associated with aggressive prostate cancer.

Studies of the Decipher genetic test have evaluated its correlation with tumor characteristics (Den, et al., 2016; Klein, et al., 2016) and reported on the use of this gene panel to predict biochemical recurrence, metastatic progression, and disease-specific survival after radical prostatectomy with or without external beam radiotherapy (Ehro, et al., 2013; Den, et al., 2013; Cooperberg, et al., 2015; Ross, et al., 2014; Klein, et al., 2015; Karnes, et al., 2013; Den, et al., 2014; Den, et al., 2015; Lee, et al., 2016; Klein, et al., 2016; Glass, et al., 2016; Freedland, et al., 2016; Ross, et al., 2016). The impact of Decipher was evaluated in a clinical utility study where 21 uro-oncologists were presented 24 patient cases (12 potential candidates for adjuvant and 12 for salvage external beam radiation therapy) and were asked for treatment recommendations with and without information from the genetic test (Badani, et al., 2013). The recommendation changed in 43% of the adjuvant cases and 53% in the salvage setting, suggesting a
potentially significant impact on treatment decisions after radical prostatectomy. Michalopoulos, et al. (2014) reported that the Decipher genomic classifier was useful in the clinic when used as a part of the risk stratification in recommending adjuvant radiation to patients with high-risk pathologic features. In that study, 43% of patients shifted to observation based on information of Decipher genomic classifier after radical prostatectomy. Nguyen, et al. (2015) examined how the results of the Decipher test altered recommendations of radiation oncologists and urologists for adjuvant treatment of 10 patients status post RP for prostate cancer. Using clinical information alone, observation was recommended in 42% of decisions made by urologists versus 23% by radiation oncologists (P < .0001). The GC test results altered 35% and 45% of treatment recommendations made by radiation oncologists and urologists, respectively. Badani, et al. (2015) reported on a study where 51 urologists provided treatment recommendations for patients with high-risk prostate cancer with and without Decipher GC test results. Each urologist was asked to provide treatment recommendations on 10 cases randomly drawn from a pool of 100 case histories. Without Decipher GC test result knowledge, observation was recommended for 57% (n = 303), adjuvant radiation therapy (ART) for 36% (n = 193) and other treatments for 7% (n = 34) of patients. Overall, 31% (95% CI: 27-35%) of treatment recommendations changed with knowledge of the Decipher GC test results. However, the long-term impact of these changes in management is unknown (Bostrom, et al., 2015).

Spratt, et al. (2017) performed an individual patient-level metaanalysis of the performance of the Decipher genomic classifier in high-risk men after prostatectomy to predict the development of metastatic disease. MEDLINE, EMBASE, and the Decipher genomic resource information database were searched for published reports between 2011 and 2016 of men treated by prostatectomy that assessed the benefit of the Decipher test. Multivariable Cox proportional hazards models fit to individual patient data were performed; meta-analyses were conducted by pooling the study-specific hazard ratios (HRs) using random-effects modeling. Extent of heterogeneity between studies was determined with the I(2) test. Five studies (975 total patients, and
855 patients with individual patient-level data were eligible for analysis, with a median follow-up of 8 years. Of the total cohort, 60.9%, 22.6%, and 16.5% of patients were classified by Decipher as low, intermediate, and high risk, respectively. The 10-year cumulative incidence metastases rates were 5.5%, 15.0%, and 26.7% (P < .001), respectively, for the three risk classifications. Pooling the study-specific Decipher HRs across the five studies resulted in an HR of 1.52 (95% CI, 1.39 to 1.67; I(2) = 0%) per 0.1 unit. In multivariable analysis of individual patient data, adjusting for clinicopathologic variables, Decipher remained a statistically significant predictor of metastasis (HR, 1.30; 95% CI, 1.14 to 1.47; P < .001) per 0.1 unit. The C-index for 10-year distant metastasis of the clinical model alone was 0.76; this increased to 0.81 with inclusion of Decipher. The authors concluded that the Decipher test can improve prognostication of patients postprostatectomy. The authors stated that future study of how to best incorporate genomic testing in clinical decision-making and subsequent treatment recommendations is warranted.

Dalela, et al. (2017) aimed to develop and internally validate a risk-stratification tool incorporating the Decipher score, along with routinely available clinicopathologic features, to identify patients who would benefit the most from postoperative adjuvant radiation therapy. Patient and Methods Our cohort included 512 patients with prostate cancer treated with radical prostatectomy at one of four US academic centers between 1990 and 2010. All patients had ≥ pT3a disease, positive surgical margins, and/or pathologic lymph node invasion. Multivariable Cox regression analysis tested the relationship between available predictors (including Decipher score) and clinical recurrence (CR), which were then used to develop a novel risk-stratification tool. Overall, 21.9% of patients received adjuvant radiotherapy. Median follow-up in censored patients was 8.3 years. The 10-year CR rate was 4.9% vs. 17.4% in patients treated with adjuvant radiotherapy versus initial observation (P < .001). Pathologic T3b/T4 stage, Gleason score 8-10, lymph node invasion, and Decipher score > 0.6 were independent predictors of CR (all P < .01). The cumulative number of risk factors was 0, 1, 2, and 3 to 4 in 46.5%, 28.9%, 17.2%, and 7.4% of patients, respectively. Adjuvant radiotherapy was associated with
decreased CR rate in patients with two or more risk factors (10-year CR rate 10.1% in aRT v 42.1% in initial observation; P = .012), but not in those with fewer than two risk factors (P = 0.18). The investigators concluded that, using the new model to indicate adjuvant radiotherapy might reduce overtreatment, decrease unnecessary adverse effects, and reduce risk of CR in the subset of patients (approximately 25% of all patients with aggressive pathologic disease in our cohort) who benefit from this therapy.

In a review of genomic predictors of outcome in prostate cancer, Bostrom, et al. (2015) noted that the Decipher test, like other gene panels (Prolaris, Oncotype DX Genomic Prostate Score) have been evaluated in terms of potential prognostic value after RP. The future will tell if this additional information is considered sufficient by the urologic and prostate cancer patients to change practice (Bostrom, et al., 2015; Nguyen, et al., 2015). Bostrom, et al. (2015) commented: “Although clinical studies have suggested potential benefits with these tests, real clinical use and long-term data are needed to judge the added value.”

NCCN guidelines on prostate cancer (NCCN, 2016) state: “These molecular tests listed [Decipher, Ki-67, Oncotype Dx Prostate, Prolaris, ProMark, PTEN] have been developed with extensive industry support, guidance, involvement and have been marketed under the less rigorous FDA regulatory pathway for biomarkers. Although full assessment of their clinical utility requires prospective, randomized clinical trials, which are unlikely to be done, the panel believes that men with clinically localized disease may consider the use of tumor-based molecular assays at this time. Future comparative effectiveness research may allow these tests and others like them to gain additional evidence regarding their utility for better risk stratification of men with prostate cancer.”

Moschini, et al. (2016) commented that, although these recommendations for Decipher have been reported in the National Comprehensive Cancer Network (NCCN) guidelines, clinical use and long-term data are needed to judge the real added value.
Guidelines on localized prostate cancer from the American Urologic Association (Sanda, et al., 2017) state based upon expert opinion that, among most low-risk localized prostate cancer patients, Decipher and other tissue based genomic biomarkers have not shown a clear role in the selection of candidates for active surveillance. The guidelines also indicate that tissue based genomic biomarkers are not necessary for followup. The guidelines state that the Decipher test has not been validated as providing substantial benefit in the active surveillance population.

**miRNAs for prostate cancer**

Maugeri-Sacca et al (2013) stated that prostate cancer is one of the most common causes of cancer-related death. The management of prostate cancer patients has become increasingly complex, consequently calling on the need for identifying and validating prognostic and predictive biomarkers. Growing evidence indicates that microRNAs play a crucial role in the pathobiology of neoplastic diseases. The deregulation of the cellular "miRNome" in prostate cancer has been connected with multiple tumor-promoting activities such as aberrant activation of growth signals, anti-apoptotic effects, pro-metastatic mechanisms, alteration of the androgen receptor pathway, and regulation of the cancer stem cell phenotype. With the elucidation of molecular mechanisms controlled by microRNAs, investigations have been conducted in an attempt to exploit these molecules in the clinical setting. Moreover, the multi-faceted biological activity of microRNAs makes them an attractive candidate as anti-cancer agents. This review summarized the current knowledge on microRNA deregulation in prostate cancer, and the rationale underlying their exploitation as cancer biomarkers and therapeutics.

Yu and Xia (2013) discussed the novel biomarkers of microRNAs in prostate cancer. The literatures about microRNAs and prostate cancer cited in this review were obtained mainly from PubMed published in English from 2004 to 2012. Original articles regarding the novel role of microRNAs in prostate cancer were selected. MicroRNAs play an important role in prostate cancer such as cell differentiation, proliferation, apoptosis, and invasion.
 Especially microRNAs correlate with prostate cancer cell epithelial-mesenchymal transition (EMT), cancer stem cells (CSCs), drug sensitivity, cancer microenvironment, energy metabolism, androgen independence transformation, and diagnosis prediction. The authors concluded that microRNAs are involved in various aspects of prostate cancer biology. Moreover, they state that the role of microRNA in the initiation and development of prostate cancer deserves further study.

Chiam et al (2014) noted that epigenome alterations are characteristic of nearly all human malignancies and include changes in DNA methylation, histone modifications and microRNAs (miRNAs). However, what induces these epigenetic alterations in cancer is largely unknown and their mechanistic role in prostate tumorigenesis is just beginning to be evaluated. Identification of the epigenetic modifications involved in the development and progression of prostate cancer will not only identify novel therapeutic targets but also prognostic and diagnostic markers. This review focused on the use of epigenetic modifications as biomarkers for prostate cancer.

Furthermore, the National Comprehensive Cancer Network’s clinical practice guideline on “Prostate cancer” (Version 1.2014) does not mention the use of RNA/microRNA biomarker as a management tool.

Galectin-3

There has been emerging evidence for galectin-3 in the pathogenesis and progression of prostate cancer. However, there is insufficient evidence for its impact in screening, diagnosis or management. National Comprehensive Cancer Network’s clinical practice guideline on “Prostate cancer” (Version 1.2015) as well as its Biomarkers Compendium has no recommendation for galectin-3 in prostate cancer.

MLH1 Promotor Methylation

Metcalf et al (2014) stated that colorectal cancer (CRC) that displays high microsatellite instability (MSI-H) can be caused by
either germline mutations in mismatch repair (MMR) genes, or non-inherited transcriptional silencing of the MLH1 promoter. A correlation between MLH1 promoter methylation, specifically the 'C' region, and BRAF V600E status has been reported in CRC studies. Germline MMR mutations also greatly increase risk of endometrial cancer (EC), but no systematic review has been undertaken to determine if these tumor markers may be useful predictors of MMR mutation status in EC patients. Endometrial cancer cohorts meeting review inclusion criteria encompassed 2,675 tumors from 20 studies for BRAF V600E, and 447 tumors from 11 studies for MLH1 methylation testing. BRAF V600E mutations were reported in 4/2,675 (0.1 %) endometrial tumors of unknown MMR mutation status, and there were 7/823 (0.9 %) total sequence variants in exon 11 and 27/1012 (2.7 %) in exon 15. Promoter MLH1 methylation was not observed in tumors from 32 MLH1 mutation carriers, or for 13 MSH2 or MSH6 mutation carriers. MMR mutation-negative individuals with tumor MLH1 and PMS2 IHC loss displayed MLH1 methylation in 48/51 (94 %) of tumors. These researchers had also detailed specific examples that showed the importance of MLH1 promoter region, assay design, and quantification of methylation. The authors concluded that this review showed that BRAF mutations occurs so infrequently in endometrial tumors they can be discounted as a useful marker for predicting MMR-negative mutation status, and further studies of endometrial cohorts with known MMR mutation status are needed to quantify the utility of tumor MLH1 promoter methylation as a marker of negative germline MMR mutation status in EC patients.

Furthermore, UpToDate reviews on "Endometrial carcinoma: Clinical features and diagnosis" (Chen ad Berek, 2015) and "Overview of endometrial carcinoma" (Plaxe and Mundt, 2015) as well as NCCN’s clinical practice guideline on “Uterine neoplasms” (Version 2.2015) do not mention testing of MLH1 promoter methylation.

p16

p16 is a tumor suppressor gene that regulates cellular proliferation and growth by acting as a cyclin-dependent kinase 4
(CDK4) inhibitor (Chen, et al. 2006). This test determines if a patient has a p16 gene mutation, indicating a predisposition for melanoma and pancreatic cancer.

Chung et al (2014) noted that although p16 protein expression, a surrogate marker of oncogenic human papillomavirus (HPV) infection, is recognized as a prognostic marker in oropharyngeal squamous cell carcinoma (OPSCC), its prevalence and significance have not been well-established in cancer of the oral cavity, hypopharynx, or larynx, collectively referred as non-OPSCC, where HPV infection is less common than in the oropharynx. p16 expression and high-risk HPV status in non-OPSCCs from RTOG 0129, 0234, and 0522 studies were determined by immunohistochemistry (IHC) and in-situ hybridization (ISH). Hazard ratios from Cox models were expressed as positive or negative, stratified by trial, and adjusted for clinical characteristics. p16 expression was positive in 14.1 % (12 of 85), 24.2 % (23 of 95), and 19.0 % (27 of 142) and HPV ISH was positive in 6.5 % (6 of 93), 14.6 % (15 of 103), and 6.9 % (7 of 101) of non-OPSCCs from RTOG 0129, 0234, and 0522 studies, respectively. Hazard ratios for p16 expression were 0.63 (95 % CI: 0.42 to 0.95; p = 0.03) and 0.56 (95 % CI: 0.35 to 0.89; p = 0.01) for PFS and OS, respectively. Comparing OPSCC and non-OPSCC, patients with p16-positive OPSCC have better PFS and OS than patients with p16-positive non-OPSCC, but patients with p16-negative OPSCC and non-OPSCC have similar outcomes. The authors concluded that similar to results in patients with OPSCC, patients with p16-negative non-OPSCC have worse outcomes than patients with p16-positive non-OPSCC, and HPV may also have a role in outcome in a subset of non-OPSCC. However, these investigators stated that further development of a p16 IHC scoring system in non-OPSCC and improvement of HPV detection methods are needed before broad application in the clinical setting; they noted that additional research using multi-modality testing in non-OPSCC and development of more accurate HPV testing are indicated.

*MUC5AC*

Ruzzenente et al (2014) stated that mucin 5AC (MUC5AC) is a
glycoprotein found in different epithelial cancers, including biliary tract cancer (BTC). These researchers examined the role of MUC5AC as serum marker for BTC and its prognostic value after operation with curative intent. From January 2007 to July 2012, a quantitative assessment of serum MUC5AC was performed with enzyme-linked immunoassay in a total of 88 subjects. Clinical and biochemical data (including CEA and Ca 19-9) of 49 patients with BTC were compared with a control population that included 23 patients with benign biliary disease (BBD) and 16 healthy control subjects (HCS). Serum MUC5AC was greater in BTC patients (mean 17.93 ± 10.39 ng/ml) compared with BBD (mean 5.95 ± 5.39 ng/ml; p < 0.01) and HCS (mean 2.74 ± 1.35 ng/ml; p < 0.01). Multi-variate analysis showed that MUC5AC was related with the presence of BTC compared with Ca 19-9 and CEA: p < 0.01, p = 0.080, and p = 0.463, respectively. In the BTC group, serum MUC5AC greater than or equal to 14 ng/ml was associated with lymph-node metastasis (p = 0.050) and American Joint Committee on Cancer and International Union for Cancer Control stage IVb disease (p = 0.047). Moreover, in patients who underwent operation with curative intent, serum MUC5AC greater than or equal to 14 ng/ml was related to a worse prognosis compared with patients with lesser levels, with 3-year survival rates of 21.5 % and 59.3 %, respectively (p = .039). The authors concluded that MUC5AC could be proposed as new serum marker for BTC. Moreover, the quantitative assessment of serum MUC5AC could be related to tumor stage and long-term survival in patients with BTC undergoing operation with curative intent.

The authors stated that “Limitations of this study include the lack of data on serum levels of MUC5AC in patients with obstructive jaundice and with premalignant biliary lesions such as hepatolithiasis, sclerosing cholangitis, and choledochal cysts …. Therefore, further studies should be addressed to clarify the diagnostic value of serum MUC5AC also in patients with obstructive jaundice and with premalignant lesions …. Our data should be confirmed by well-designed large-scale prospective studies”.

Furthermore, NCCN’s clinical practice guideline on “Hepatobiliary
cancers” (Version 2.2015) does not mention mucin 5AC (MUC5AC) as a management tool.

Tp53

In a pilot study, Erickson et al (2014) examined if tumor cells could be detected in the vagina of women with serous ovarian cancer through TP53 analysis of DNA samples collected by placement of a vaginal tampon. Women undergoing surgery for a pelvic mass were identified in the gynecologic oncology clinic. They placed a vaginal tampon before surgery, which was removed in the operating room. Cells were isolated and DNA was extracted from both the cells trapped within the tampon and the primary tumor. In patients with serous carcinoma, the DNA was interrogated for the presence of TP53 mutations using a method capable of detecting rare mutant alleles in a mixture of mutant and wild-type DNA. A total of 33 patients were enrolled; 8 patients with advanced serous ovarian cancer were included for analysis; and 3 had a prior tubal ligation. TP53 mutations were identified in all 8 tumor samples. Analysis of the DNA from the tampons revealed mutations in 3 of the 5 patients with intact tubes (sensitivity 60 %) and in none of the 3 patients with tubal ligation. In all 3 participants with mutation detected in the tampon specimen, the tumor and the vaginal DNA harbored the exact same TP53 mutation. The fraction of DNA derived from exfoliated tumor cells ranged from 0.01 % to 0.07 %. The authors concluded that in this pilot study, DNA derived from tumor was detected in the vaginas of 60 % of patients with ovarian cancer with intact fallopian tubes. They stated that with further development, this approach may hold promise for the early detection of this deadly disease. They stated that for this method to ultimately be clinically useful, several factors should be considered -- this approach will have to be shown to be able to adequately detect early states of disease to provide sufficient lead time for an effective intervention. In this regard, one of the drawbacks of this study was that all samples were obtained from patients with late-stage cancer. Another limitation was that these researchers did not sequence the DNA from tampons from patients with benign disease. Thus, specificity could not be calculated. These investigators stated that larger studies are
needed to further validate this method and identify a more precise detection rate.

In an editorial that accompanied the afore-mentioned study, Mulch (2014) stated that “In terms of clinical utility, the sensitivity of this test may be around 60% in patients with intact tubes and with clinically obvious cancer, but we do not know what it will be in patients with less advanced disease …. However, the barrier to ovarian cancer screening is the fact that the prevalence of the disease is so low in the general population that any screening test must have an unrealistic sensitivity and specificity …. This technology shows great promise …. The technology represented here has the potential to do what other screening tests have not …. We must be careful not to endorse it until its usefulness is fully validated”.

Furthermore, NCCN’s clinical practice guideline on “Ovarian cancers” (Version 3.2014) does not mention TP53 mutation analysis as a management tool.

Zhang et al (2015) summarized the potential diagnostic value of 5 serum tumor markers in esophageal cancer. These researchers systematically searched PubMed, Embase, Chinese National Knowledge Infrastructure (CNKI) and Chinese Biomedical Database (CBM), through February 28, 2013, without language restriction. Studies were assessed for quality using QUADAS (quality assessment of studies of diagnostic accuracy). The positive likelihood ratio (PLR) and negative likelihood ratio (NLR) were pooled separately and compared with overall accuracy measures using diagnostic odds ratios (DORs) and symmetric summary receiver operating characteristic (SROC) curves. Of 4,391 studies initially identified, 44 eligible studies including 5 tumor markers met the inclusion criteria for the meta-analysis, while meta-analysis could not be conducted for 12 other tumor markers. Approximately 79.55% (35/44) of the included studies were of relatively high quality (QUADAS score greater than or equal to 7). The summary estimates of PLR, NLR and DOR for diagnosing EC were as follows: CEA, 5.94/0.76/9.26; Cyfra21-1 (a cytokeratin 19 fragment), 12.110.59/22.27; p53 antibody, 6.71/0.75/9.60; squamous cell carcinoma antigen (SCC-Ag),
7.66/0.68/12.41; and vascular endothelial growth factor C (VEGF-C), 0.74/0.37/8.12. The estimated SROC curves showed that the performance of all 5 tumor markers was reasonable. The authors concluded that the current evidence suggested that CEA, Cyfra21-1, p53, SCC-Ag and VEGF-C have a potential diagnostic value for esophageal carcinoma.

**Ki67**

There is a strong correlation between proliferation rate and clinical outcome in a variety of tumor types and measurement of cell proliferative activity is an important prognostic marker (Chen, et al., 2006). This marker correlates with flow cytometric S-phase.

There is insufficient evidence for Ki67. NCCN guidelines on breast cancer (2015) state: "The measurement of nuclear antigen, Ki-67 by IHC, gives an estimate of the tumor cells in the proliferative phase (G1, G2 and M phases) of the cell cycle. Studies have demonstrated the prognostic value of Ki-67 as a biomarker and its usefulness in predicting response and clinical outcome. One small study suggests that measurement of Ki-67 after short-term exposure to endocrine treatment may be useful to select patients resistant to endocrine therapy and those who may benefit from additional interventions. However, these data require larger analytic and clinical validation. In addition, standardization of tissue handling and processing is required to improve the reliability and value of Ki-67 testing. At this time, there is no conclusive evidence that Ki-67 alone, especially baseline Ki-67 as an individual biomarker, helps to select the type of endocrine therapy for an individual patient. Therefore, the NCCN Breast Cancer Panel does not currently recommend assessment of Ki-67."

The p16/Ki-67 Dual Stain test (CINtec PLUS) claims to detect virally induced oncogenic molecular changes in the cell through the immune cytochemical double staining of the tumor suppressor gene p16^{INK4a} and the proliferation marker Ki-67 and thereby to improve the triage of women with equivocal cytological results (Kisser, et al., 2014). The Ludwig Boltzmann Institut conducted a systematic review of studies assessing utility
of the p16/Ki-67 Dual Stain test in the triage of equivocal or mild to moderate dysplasia results in cervical cancer screening. The authors of the assessment stated that they could not identify any studies assessing clinical outcomes such as mortality or morbidity and only one high quality study assessing diagnostic accuracy of the test: the evaluation of the clinical utility of the test was therefore not possible (Kisser, et al., 2014). Consequently the test was not recommended for inclusion in the benefits catalogue of public health insurances.

Guidelines from the American Society for Clinical Oncology (2016) state: "Protein encoded by the MKI67 gene labeling index by IHC should not be used to guide choice on adjuvant chemotherapy." This is a moderate-strength recommendation based upon intermediate-quality evidence.

**Veristrat**

Mass spectrometry based proteomic profiling (such as Veristrat) is a multivariate serum protein test that uses mass spectrometry and proprietary algorithms to analyze proteins in an individual’s serum.

NCCN guidelines on non-small cell lung cancer (NCCN, 2015) recommend proteomic testing for patients with NSCLC and wild-type EGFR or with unknown EGFR status. The guidelines state that a patient with a “poor” classification should not be offered erlotinib in the second-line setting. For support, NCCN guidelines reference a study by Gregorc, et al. (2014), who reported that serum protein test status (Veristrat) is predictive of differential benefit in overall survival for erlotinib versus chemotherapy in the second-line setting, and that patients classified as likely to have a poor outcome have better outcomes on chemotherapy than on erlotinib. From Feb 26, 2008, to April 11, 2012, patients (aged ≥18 years) with histologically or cytologically confirmed, second-line, stage IIIB or IV non-small-cell lung cancer were enrolled in 14 centres in Italy. Patients were stratified according to a minimization algorithm by Eastern Cooperative Oncology Group performance status, smoking history, center, and masked pretreatment serum protein test classification, and randomly
assigned centrally in a 1:1 ratio to receive erlotinib (150 mg/day, orally) or chemotherapy (pemetrexed 500 mg/m2, intravenously, every 21 days, or docetaxel 75 mg/m2, intravenously, every 21 days). 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. Analyses were done on the per‐protocol population. Investigators randomly assigned 142 patients to chemotherapy and 143 to erlotinib, and 129 (91%) and 134 (94%), respectively, were included in the per‐protocol analysis. 88 (68%) patients in the chemotherapy group and 96 (72%) in the erlotinib group had a proteomic test classification of good. Median overall survival was 9·0 months (95% CI 6·8–10·9) in the chemotherapy group and 7·7 months (5·9–10·4) in the erlotinib group. The investigators noted a significant interaction between treatment and proteomic classification (pinteraction = 0·017 when adjusted for stratification factors; pinteraction=0·031 when unadjusted for stratification factors). The investigators found that patients with a proteomic test classification of poor had worse survival on erlotinib than on chemotherapy (hazard ratio 1·72 [95% CI 1·08–2·74], p=0·022). There was no significant difference in overall survival between treatments for patients with a proteomic test classification of good (adjusted HR 1·06 [0·77–1·46],  p=0·714). In the group of patients who received chemotherapy, the most common grade 3 or 4 toxic effect was neutropenia (19 [15%] vs one [<1%] in the erlotinib group), whereas skin toxicity (one [<1%] vs 22 [16%]) was the most frequent in the erlotinib group.

**Multiplex Testing for Myeloid Hematopathologic Disorders**

Multiplex testing/next generation sequencing can assist in the diagnosis of various myeloid hematopathologic disorders, particularly myelodysplastic syndrome (MDS). The International Consensus Working Group (ICWG) (Valent, et al., 2007) recommends that minimal diagnostic criteria for MDS include: A) Prerequisite criteria, including stable cytopenia in one or more
cell line, and exclusion of other potential disorders as a primary reason for dysplasia and/or cytopenia; B) MDS-related (decisive) criteria, including significant dysplasia, a blast count of 5-19%, and/or specific MDS cytogenetic abnormalities; and co-criteria for patients fulfilling A) but not B), including clear signs of a monoclonal population utilizing molecular markers (such as DNA mutations) or flow cytometry, or markedly reduced colony formation. In addition, many of the genes have independent prognostic value in various myeloid malignancies including ASXL1, RUNX1, ETV6, EZH2, TP53 in multivariable analysis in MDS. Other critical disease genes such as DNMT3A, CBL, IDH2, IDH1, SRSF2, ZRSR2, NRAS, U2AF1, and SF3B1 have also been shown to be independent predictors of survival in MDS as well as ASXL1, SRSF2, CBL, and IDH2 in chronic myelomonocytic leukemia (CMML), IDH1/2, EZH2, SRSF2, ASXL1 in primary myelofibrosis (PMF), and SETBP1 in atypical chronic myeloid leukemia (aCML).

ResponseDx

ResponseDX: Colon® (Response Genetics) panel utilizes testing of multiple genes including KRAS mutation, BRAF mutation, ERCC1 expression, MSI, c-Met expression, EGFR expression, VEGFR2 expression, NRAS mutation, PIK3CA mutation, and Thymidylate synthetase (Raman, et al., 2013). The test predicts disease prognosis and selects patients who might benefit from alternative therapies and aids in selection of metastatic colorectal cancer patients that might benefit from EGFR-targeted monoclonal antibody therapies.

ResponseDX:Lung® panel (Response Genetics) utilizes testing of multiple genes including ROS1 rearrangements, EGFR mutation, EML4-ALK rearrangement, ALK, ERCC1 expression, RRM1 expression, c-MET expression, TS expression, KRAS mutation, and PIK3CA mutation (Raman, et al., 2013) The test is used in patients with non-small cell lung cancer who are being considered for treatment with the tyrosine kinase inhibitor (TKI) Crizotinib.

ResponseDX:Melanoma® panel (Response Genetics) utilizes testing of multiple genes including BRAF mutation, and NRAS mutation (Raman, et al., 2013). The test is performed on
formalin-fixed, paraffin embedded (FFPE) biopsy specimen, using fluorescence in situ hybridization (FISH) and polymerase chain reaction (PCR). The test is intended for patients with melanoma who are being considered for treatment with the tyrosine kinase inhibitor (TKI) and EGFR antagonists cetuximab and panitumumab.

ResponseDX: Gastric® panel (Response Genetics) utilizes testing of multiple genes including HER2 gene amplification, ERCC1 expression, and Thymidilate Synthetase expression (Raman, et al., 2013). This is a PCR-based test performed on formalin-fixed, paraffin-embedded biopsy specimens. Amplification of the HER2 gene is associated with increased disease recurrence and a worse prognosis. ERCC1 expression predicts the best therapeutic combination of agents including platinum and select patients who might benefit from platinum-based therapies. Thymidylate synthetase (TS) expression predicts the best therapeutic combination of agents including pemetrexed or 5-FU and select patients who might benefit from pemetrexed-based therapies.

4K Score

4Kscore Test measures the blood plasma levels of four different prostate-derived kallikrein proteins [Total PSA, Free PSA, Intact PSA and Human Kallikrein2 (hK2)] and combines results in an algorithm with age, DRE (nodules, no nodules) and prior biopsy results. The result is purportedly an individual’s specific probability for finding a high-grade, Gleason score 7 or higher prostate cancer upon biopsy.

Parekh, et al. (2015) performed the first prospective evaluation of the 4Kscore in predicting Gleason ≥7 PCa in the USA. The investigators prospectively enrolled 1012 men scheduled for prostate biopsy, regardless of prostate-specific antigen level or clinical findings, from 26 US urology centers between October 2013 and April 2014. The primary outcome was Gleason ≥7 PCa on prostate biopsy. The area under the receiver operating characteristic curve, risk calibration, and decision curve analysis (DCA) were determined, along with comparisons of probability cutoffs for reducing the number of biopsies and their impact on
delaying diagnosis. Gleason ≥7 PCa was found in 231 (23%) of the 1012 patients. The investigators stated that the 4Kscore showed excellent calibration and demonstrated higher discrimination (area under the curve [AUC] 0.82) and net benefit compared to a modified Prostate Cancer Prevention Trial Risk Calculator 2.0 model and standard of care (biopsy for all men) according to DCA. A possible reduction of 30-58% in the number biopsies was identified with delayed diagnosis in only 1.3-4.7% of Gleason ≥7 PCa cases, depending on the threshold used for biopsy. Pathological assessment was performed according to the standard of care at each site without centralized review.

Stattin, et al. (2015) conducted a case-control study nested within a population-based cohort. PSA and three additional kallikreins (4KScore) were measured in cryopreserved blood from a population-based cohort in Västerbotten, Sweden. Of 40,379 men providing blood at ages 40, 50, and 60 years from 1986 to 2009, 12,542 men were followed for >15 yr. From this cohort, the Swedish Cancer Registry identified 1423 incident PCa cases, 235 with distant metastasis. Most metastatic cases occurred in men with PSA in the top quartile at age 50 yr (69%) or 60 yr (74%), whereas 20-yr risk of metastasis for men with PSA below median was low (≤0.6%). The investigators reported that, among men with PSA >2 ng/ml, a prespecified model based on four kallikrein markers significantly enhanced the prediction of metastasis compared with PSA alone. About half of all men with PSA >2 ng/ml were defined as low risk by this model and had a ≤1% 15-yr risk of metastasis. The authors concluded that, for men in their fifties, screening should focus on those in the top 10% to 25% of PSA values because the majority of subsequent cases of distant metastasis are found among these men. Testing of four kallikrein markers in men with an elevated PSA could aid biopsy decision making.

Voigt, et al. (2014) conducted a systematic review and meta-analysis to examine the aggregated results from published studies of the Kallikrein Panel. The results of the meta-analysis were used to model the Kallikrein Panel’s effect on healthcare costs. The authors reported that meta-analysis demonstrates a statistically significant improvement of 8-10% in predictive accuracy. The
authors estimated that 48% to 56% of current prostate biopsies could be avoided and that use of the Kallikrein Panel could result in annual US savings approaching $1 billion.

Konety, et al. (2015) conducted a clinical utility study to assess the influence of the 4Kscore Test on the decision to perform prostate biopsies in men referred to urologists for abnormal PSA and/or DRE results. The study population included 611 patients seen by 35 academic and community urologists in the United States. Urologists ordered the 4Kscore Test as part of their assessment of men referred for abnormal PSA and/or DRE test results. Results for the patients were stratified into low risk (< 7.5%), intermediate risk (7.5%-19.9%), and high risk (≥ 20%) for aggressive prostate cancer. The investigators reported that the 4Kscore Test results influenced biopsy decisions in 88.7% of the men. Performing the 4Kscore Test resulted in a 64.6% reduction in prostate biopsies in patients; the actual percentage of cases not proceeding to biopsy were 94.0%, 52.9%, and 19.0% for men who had low-, intermediate-, and high-risk 4Kscore Test results, respectively. A higher 4Kscore Test was associated with greater likelihood of having a prostate biopsy (P < 0.001). The investigators reported that, among the 171 patients who had a biopsy, the 4Kscore risk category is strongly associated with biopsy pathology.

Lin, et al. (2016) sought to evaluate the utility of the 4Kscore in predicting the presence of high-grade cancer in men on active surveillance. Plasma collected before the first and subsequent surveillance biopsies was assessed for 718 men prospectively enrolled in the multi-institutional Canary PASS trial. Biopsy data were split 2:1 into training and test sets. The investigators developed statistical models that included clinical information and either the 4Kpanel or serum prostate-specific antigen (PSA). The endpoint was reclassification to Gleason ≥7. The investigators used receiver operating characteristic (ROC) curve analyses and area under the curve (AUC) to assess discriminatory capacity, and decision curve analysis (DCA) to report clinical net benefit. Significant predictors for reclassification were 4Kpanel (odds ratio [OR] 1.54, 95% confidence interval [CI] 1.31-1.81) or PSA (OR 2.11, 95% CI
1.53-2.91), ≥20% cores positive (OR 2.10, 95% CI 1.33-3.32), two or more prior negative biopsies (OR 0.19, 95% CI 0.04-0.85), prostate volume (OR 0.47, 95% CI 0.31-0.70), and body mass index (OR 1.09, 95% CI 1.04-1.14). ROC curve analysis comparing 4K and base models indicated that the 4Kpanel improved accuracy for predicting reclassification (AUC 0.78 vs 0.74) at the first surveillance biopsy. Both models performed comparably for prediction of reclassification at subsequent biopsies (AUC 0.75 vs 0.76). In DCA, both models showed higher net benefit compared to biopsy-all and biopsy-none strategies. Limitations include the single cohort nature of the study and the small numbers; results should be validated in another cohort before clinical use.

Guidelines from the National Comprehensive Cancer Network (NCCN, 2016) lists the 4Kscore nonpreferentially among a number of tests (i.e., the percent free PSA and the Prostate Health Index (PHI)) that can be considered for patients prior to biopsy and among several tests (i.e., percent free PSA, PHI, PCA3 and ConfirmMDx) for those with prior negative biopsy for men thought to be at higher risk for clinically significant prostate cancer. The NCCN guidelines state that the 4Kscore cannot be recommended over other tests (i.e., the percent free PSA, the Prostate Health Index (PHI)), The NCCN guidelines explain that head-to-head comparisons have been performed in Europe for some of these tests, performed individually or in combinations in the initial or repeat biopsy settings, but sample sizes were small and results varied. The NCCN guidelines stated that the optimal order of biomarker tests and imaging is unknown, and that it remains unclear how to interpret multiple tests in individual patients, especially when results are contradictory. The panel states that it is important for patients and their urologists to understand, however, that no cutoff threshold has been established for the 4KScore.

Recommendations from Memorial Sloan Kettering (Vickers, et al., 2016) state that in biopsy-naive men with PSA ≥3 ng/mL, prostate MRI is the strongest independent predictor of clinically significant prostate cancer, but “[a]s evidence continues to build, we believe that prostate MRI may emerge as a valuable tool to reduce overdiagnosis of PCa, most likely in concert with newer
biomarkers, such as the Prostate Health Index, the 4Kscore, and single nucleotide polymorphism panels.

A 2016 MolDx assessment of the 4KScore concluded that "the intended use population has been inadequately validated; the 4Kscore model has continuously changed; the model has been recurrently tested on potentially inappropriate patients (PSA > 10) and patients with inadequate biopsy sampling; it is unclear how much the hK2 and possibly intact PSA contribute to the model; the value of the 4Kscore model/algorithm is fraught with statistical hypothesis and not prospective outcomes or concordance in a defined patient population likely to be considered for biopsy (eg: PSA 3-10 ng/mL); assumptions are made that no harm will come to following young men with unknown low grade prostate cancer (not on AS); there is significant difficulty equating the model used in the Swedish study to the presently proposed formula; and the incidence of clinically diagnosable prostate cancer in patients with low risk by the model/algorithm at 10 years is very concerning."

**ConfirmMDx**

ConfirmMDx (MDxHealth, Irvine, CA) is an epigenetic assay using multiplex polymerase chain reaction (PCR) to measure DNA methylation of gene regions that are associated with cancer. It is designed to distinguish patients with prostate cancer who have a true-negative biopsy from those who may have occult cancer. The test supposedly helps urologists rule-out prostate cancer-free men from undergoing unnecessary repeat biopsies and, helps rule-in high-risk patients who may require repeat biopsies and potential treatment. However, there is inadequate evidence to support the clinical value of ConfirmMDx in patients with prostate cancer.

Stewart et al (2013) reported that ConfirmMDx, a multiplex quantitative methylation specific polymerase chain reaction assay determining the methylation status of GSTP1, APC and RASSF1, was strongly associated with repeat biopsy outcome up to 30 months after initial negative biopsy in men with suspicion of prostate cancer. The investigators blindly tested archived
prostate biopsy needle core tissue samples of 498 subjects from the United Kingdom and Belgium with histopathologically negative prostate biopsies, followed by positive (cases) or negative (controls) repeat biopsy within 30 months. Clinical performance of the epigenetic marker panel, emphasizing negative predictive value, was assessed and cross-validated. Multivariate logistic regression was used to evaluate all risk factors. The epigenetic assay performed on the first negative biopsies of this retrospective review cohort resulted in a negative predictive value of 90 % (95 % CI: 87 to 93). In a multivariate model correcting for patient age, prostate specific antigen, digital rectal examination and first biopsy histopathological characteristics the epigenetic assay was a significant independent predictor of patient outcome (OR 3.17, 95 % CI: 1.81 to 5.53). The investigators stated that adding this epigenetic assay could improve the prostate cancer diagnostic process and decrease unnecessary repeat biopsies.

Partin et al (2014) reported that the ConfirmMDx epigenetic assay was a significant, independent predictor of prostate cancer detection in a repeat biopsy collected an average of 13 months after an initial negative result. The investigators evaluated the archived, cancer negative prostate biopsy core tissue samples of 350 subjects from a total of 5 urological centers in the United States. All subjects underwent repeat biopsy within 24 months with a negative (controls) or positive (cases) histopathological result. Centralized blinded pathology evaluation of the 2 biopsy series was performed in all available subjects from each site. Biopsies were epigenetically profiled for GSTP1, APC and RASSF1 relative to the ACTB reference gene using quantitative methylation specific polymerase chain reaction. Pre-determined analytical marker cutoffs were used to determine assay performance. Multivariate logistic regression was used to evaluate all risk factors. The epigenetic assay resulted in a negative predictive value of 88 % (95 % CI: 85 to 91). In multivariate models correcting for age, prostate specific antigen, digital rectal examination, first biopsy histopathological characteristics and race the test proved to be the most significant independent predictor of patient outcome (OR 2.69, 95 % CI: 1.60 to 4.51). The investigators stated that adding this epigenetic
assay to other known risk factors may help decrease unnecessary repeat prostate biopsies.

Wu and colleagues (2011) noted that PSA screening has low specificity. Assessment of methylation status in body fluids may complement PSA screening if the test has high specificity. The purpose of this study was to conduct a meta-analysis of the sensitivity and specificity for prostate cancer detection of glutathione-s-transferase-π (GSTP1) methylation in body fluids (plasma, serum, whole blood, urine, ejaculate, and prostatic secretions). These researchers conducted a comprehensive literature search on Medline (PubMed). They included studies if they met all 4 of the following criteria: (i) measurement of DNA methylation in body fluids; (ii) a case-control or case-only design; (iii) publication in an English journal; and (iv) adult subjects. Reviewers conducted data extraction independently using a standardized protocol. A total of 22 studies were finally included in this paper. Primer sequences and methylation method in each study were summarized and evaluated using meta-analyses. This paper represented a unique cross-disciplinary approach to molecular epidemiology. The pooled specificity of GSTP1 promoter methylation measured in plasma, serum, and urine samples from negative-biopsy controls was 0.89 (95 % CI: 0.80 to 0.95). Stratified analyses consistently showed a high specificity across different sample types and methylation methods (include both primer sequences and location). The pooled sensitivity was 0.52 (95 % CI: 0.40 to 0.64). The authors concluded that the pooled specificity of GSTP1 promoter methylation measures in plasma, serum, and urine was excellent and much higher than the specificity of PSA. The sensitivity of GSTP1 was modest, no higher than that of PSA. They stated that these findings suggested that measurement of GSTP1 promoter methylation in plasma, serum, or urine samples may complement PSA screening for prostate cancer diagnosis.

Van Neste et al (2012a) from the MDxHealth stated that PSA-directed prostate cancer screening leads to a high rate of false-positive identifications and an unnecessary biopsy burden. Epigenetic biomarkers have proven useful, exhibiting frequent and abundant inactivation of tumor suppressor genes through
such mechanisms. An epigenetic, multiplex PCR test for prostate cancer diagnosis could provide physicians with better tools to help their patients. Biomarkers like GSTP1, APC and RASSF1 have demonstrated involvement with prostate cancer, with the latter 2 genes playing prominent roles in the field effect. The epigenetic states of these genes can be used to assess the likelihood of cancer presence or absence. An initial test cohort of 30 prostate cancer-positive samples and 12 cancer-negative samples was used as basis for the development and optimization of an epigenetic multiplex assay based on the GSTP1, APC and RASSF1 genes, using methylation specific PCR (MSP). The effect of prostate needle core biopsy sample volume and age of formalin-fixed paraffin-embedded (FFPE) samples was evaluated on an independent follow-up cohort of 51 cancer-positive patients. Multiplexing affects copy number calculations in a consistent way per assay. Methylation ratios are therefore altered compared to the respective singleplex assays, but the correlation with patient outcome remains equivalent. In addition, tissue-biopsy samples as small as 20 μm can be used to detect methylation in a reliable manner. The age of FFPE-samples does have a negative impact on DNA quality and quantity. The authors concluded that the developed multiplex assay appears functionally similar to individual singleplex assays, with the benefit of lower tissue requirements, lower cost and decreased signal variation. This assay can be applied to small biopsy specimens, down to 20 microns, widening clinical applicability. Increasing the sample volume can compensate the loss of DNA quality and quantity in older samples.

Van Neste et al (2012b) noted that prostate cancer is the most common cancer diagnosis in men and a leading cause of death. Improvements in disease management would have a significant impact and could be facilitated by the development of biomarkers, whether for diagnostic, prognostic, or predictive purposes. The blood-based prostate biomarker PSA has been part of clinical practice for over 2 decades, although it is surrounded by controversy. While debates of usefulness are ongoing, alternatives should be explored. Particularly with recent recommendations against routine PSA-testing, the time is ripe to explore promising biomarkers to yield a more efficient and
accurate screening for detection and management of prostate cancer. Epigenetic changes, more specifically DNA methylation, are among the most common alterations in human cancer. These changes are associated with transcriptional silencing of genes, leading to an altered cellular biology. One gene in particular, GSTP1, has been widely studied in prostate cancer. Thus, a meta-analysis has been conducted to examine the role of this and other genes and the potential contribution to prostate cancer management and screening refinement. More than 30 independent, peer-reviewed studies have reported a consistently high sensitivity and specificity of GSTP1 hyper-methylation in prostatectomy or biopsy tissue. The meta-analysis combined and compared these results. The authors concluded that GSTP1 methylation detection can serve an important role in prostate cancer management. The meta-analysis clearly confirmed a link between tissue DNA hyper-methylation of this and other genes and prostate cancer. They stated that detection of DNA methylation in genes, including GSTP1, could serve an important role in clinical practice.

Andres et al (2013) synthesized the principal advances in the field of the study of epigenetics and specifically DNA methylation regarding the diagnosis of urological neoplasms. Review of the literature (PubMed, MEDLINE y COCHRANE) on the study of DNA methylation in urological neoplasms (prostate cancer, bladder cancer, renal cancer and testicular cancer), considering all the studies published up to January 2013 was carried out. It was possible to determine the state of methylation of many genes in tumor samples. When these were compared with healthy tissue samples, it was possible to define the specific aberrant methylation patterns for each type of tumor. The study and definition of specific abnormal methylation patterns of each type of tumor is a tool having potential utility for diagnosis, evaluation, prediction of prognosis and treatment of the different forms of genito-urinary cancer. The analysis of gene methylation in urine after micturition or post-prostatic massage urine, semen, in the wash plasma or fluid from prostatic biopsies may allow early detection of bladder, prostate, renal and testicular cancer. In each of the neoplasms, an epigenetic signature that may be detected in the DNA has been identified, obtained from very
scarce or not at all invasive specimens, with potential in the
diagnosis and evaluation of prognosis. Validation of these studies
will confirm the accuracy, effectiveness, and reproducibility of the
results available up to now. Criteria have still not been developed
that determine if a gene panel provides sufficient information in
the health care practice to guide an unequivocal diagnosis or
therapeutic conduct. More studies are needed to compare
sensitivity, specificity, positive- and negative-predictive values of
the test in each case. Multi-center studies analyzing the real
reproducibility of these results in a clinical setting also do not
exist. The authors concluded that the study of aberrant DNA
methylation in biological specimens of patients has an enormous
potential for the early diagnosis and screening of genitourinary
neoplasms. They stated that more studies are needed to define
the series of genes that would mean unequivocal signatures of
malignancy. This methodology also has potential when defining
prognostic groups and potential of response to different therapies

Lin et al (2013) prostate cancer is the second leading cause of
cancer death among men worldwide, and not all men diagnosed
with prostate cancer will die from the disease. A critical
challenge, therefore, is to distinguish indolent prostate cancer
from more advanced forms to guide appropriate treatment
decisions. These investigators used Enhanced Reduced
Representation Bisulfite Sequencing, a genome-wide high-
coverage single-base resolution DNA methylation method to
profile seven localized prostate cancer samples, 7 matched
benign prostate tissues, and 6 aggressive castration-resistant
prostate cancer (CRPC) samples. They integrated these data with
RNA-seq and whole-genome DNA-seq data to comprehensively
characterize the prostate cancer methylome, detect changes
associated with disease progression, and identify novel candidate
prognostic biomarkers. The analyses revealed the correlation of
cytosine guanine dinucleotide island (CGI)-specific hyper-
methylation with disease severity and association of certain
breakpoints (deletion, tandem duplications, and inter-
chromosomal translocations) with DNA methylation.
Furthermore, integrative analysis of methylation and single-
nucleotide polymorphisms (SNPs) uncovered widespread allele-
specific methylation (ASM) for the first time in prostate cancer.
These researchers found that most DNA methylation changes occurred in the context of ASM, suggesting that variations in tumor epigenetic landscape of individuals are partly mediated by genetic differences, which may affect prostate cancer disease progression. They further selected a panel of 13 CGIs demonstrating increased DNA methylation with disease progression and validated this panel in an independent cohort of 20 benign prostate tissues, 16 prostate cancer cases, and 8 aggressive CRPCs. The authors concluded that these results warrant clinical evaluation in larger cohorts to help distinguish indolent prostate cancer from advanced disease.

Wojno and colleagues (2014) reported on an observational study that suggests that the ConfirmMDx may reduce biopsy rates in persons suspected of having prostate cancer. The investigators noted that the diagnosis of prostate cancer (PCa) is dependent on histologic confirmation in biopsy core tissues. The biopsy procedure is invasive, puts the patient at risk for complications, and is subject to significant sampling errors. The authors stated that an epigenetic test that uses methylation-specific PCR to determine the epigenetic status of the PCa-associated genes GSTP1, APC, and RASSF1 has been clinically validated and is used in clinical practice to increase the negative predictive value (NPV) in men with no history of PCa compared with standard histopathology. The investigators stated that such information can help to avoid unnecessary repeat biopsies. The investigators posited that the repeat biopsy rate may provide preliminary clinical utility evidence in relation to this assay's potential impact on the number of unnecessary repeat prostate biopsies performed in U.S. urology practices. The investigators stated that the purpose of this preliminary study was to quantify the number of repeat prostate biopsy procedures to demonstrate a low repeat biopsy rate for men with a history of negative histopathology who received a negative epigenetic assay result on testing of the residual prostate tissue. In this field observation study, practicing urologists used the ConfirmMDx for PCa to evaluate cancer-negative men considered at risk for PCa. The authors stated that his test has been previously validated in 2 blinded multi-center studies that showed the superior NPV of the epigenetic test over standard histopathology for cancer detection.
in prostate biopsies. A total of 5 clinical urology practices that had ordered a minimum of 40 commercial epigenetic test requisitions for patients with previous, cancer-negative biopsies over the course of the previous 18 months were contacted to assess their interest to participate in the study. Select demographic and prostate-screening parameter information, as well as the incidence of repeat biopsy, specifically for patients with a negative test result, was collected and merged into 1 collective database. All men from each of the 5 sites who had negative assay results were included in the analysis. A total of 138 patients were identified in these urology practices and were included in the analysis. The median age of the men was 63 years, and the current median serum PSA level was 4.7 ng/ml. Repeat biopsies had been performed in 6 of the 138 (4.3 %) men with a negative epigenetic assay result, in whom no evidence of cancer was found on histopathology. The authors concluded that in this study, a low rate of repeat prostatic biopsies was observed in the group of men with previous histopathologically negative biopsies who were considered to be at risk for harboring cancer. The data suggested that patients managed using the ConfirmMDx for PCa negative results had a low rate of repeat prostate biopsies. Moreover, they stated that these results warrant a large, controlled, prospective study to further evaluate the clinical utility of the epigenetic test to lower the unnecessary repeat biopsy rate.

The PASCUAL Study is a 600 patient randomized, controlled prospective study to track the clinical utility of the ConfirmMDx assay in U.S. urologic practices for the management of patients with a previous histopathologically cancer-negative biopsy, but clinical risk factors suggesting the need for a repeat biopsy (MDxHealth, 2016). The study, initiated in 2014, will compare the rate of repeat biopsies under the standard of care to the rate of repeat biopsies in patients managed with ConfirmMDx test results. The study is expected to be completed in 2017.

A review by Bostrom et al (2015) stated that commercially available epigenetic ConfirmMDx may be of value when repeat biopsies are considered after negative initial prostate biopsies. The review concluded: "Many new genetic-based tests are newly
available or in late stages of clinical development, with potential applications in PCa decisions ranging from the need for repeat biopsy to initial treatment selection, decisions about secondary therapy, and selection of treatment for advanced disease. Greater understanding of the potential long-term benefits and limitations of these tests is important, and how exactly they should be used in clinical practice to optimize decision making must be the subject of future prospective studies”.

A HealthPACT assessment of ConfirmMDX (Foerster et al, 2013) concluded: "From the evidence base available, prostate cancer detection using DNA methylation assays appears to offer some benefit over existing methods of diagnosis. In particular, the high NPVs and the low rates of false-negatives observed indicate that DNA methylation assays may provide a means of reducing the number of healthy men incorrectly diagnosed and subjected to unnecessary biopsy; although, high-quality comparative studies are needed before this can be truly determined. Further studies investigating the effects of prostate cancer detection using DNA methylation compared with conventional techniques on overall patient survival are also required”.

Nguyen and colleagues (2015) stated that over the past several years, multiple biomarkers designed to improve prostate cancer risk stratification have become commercially available, while others are still being developed. In this review, these researchers focused on the evidence supporting recently reported biomarkers, with a focus on gene expression signatures. Many recently developed biomarkers are able to improve upon traditional risk assessment at nearly all stages of disease. ConfirmMDx uses gene methylation patterns to improve detection of clinically significant cancer following negative biopsy. Both the Prolaris and Oncotype DX Genomic Prostate Score tests can improve risk stratification following biopsy, especially among men who are eligible for active surveillance. Prolaris and the Decipher genomic classifier have been associated with risk of adverse outcome following prostatectomy, while Oncotype DX is being studied in this setting. Finally, recent reports of the association of androgen receptor-V7 in circulating tumor cells
with resistance to enzalutamide and abiraterone raised the possibility of extending the use of genetic biomarkers to advanced disease. The authors concluded that with the development of multiple genetic expression panels in prostate cancer, careful study and validation of these tests and integration into clinical practice will be critical to realizing the potential of these tools.

Guidelines from 2015 from the National Comprehensive Cancer Network on prostate cancer screening (NCCN, 2015) concluded, regarding ConfirmMDx: "Despite the good NPV of this test, the panel noted that the study population included only patients who were ultimately selected for repeat biopsy. Furthermore, the panel noted that the NPV of first negative biopsy alone is already in the range of 75% to 80% and questioned the true value added by the test results. Therefore, until prospective data for this test or data comparing this test to other tests are available, the panel does not recommend its use".

Guidelines from the National Comprehensive Cancer Network (NCCN, 2016) recommend ConfirmMDx nonpreferentially among several markers (percent free PSA, PHI, 4KScore, PCA3 and ConfirmMDx) for men contemplating repeat biopsy because the assay may identify individuals at higher risk of prostate cancer diagnosis on repeat biopsy. The guidelines note that direct comparisons have been performed for some of these tests, used independently or in combinations, in the initial or repeat biopsy settings, but sample sizes were small and results varied. Therefore, the NCCN panel concluded that no biomarker test can be recommended over any other at this time. The NCCN panel noted the optimal order of biomarker tests and imaging is unknown; and it remains unclear how to interpret results of multiple tests in individual patients, especially when results are contradictory.

A Joint Consensus Statement from the American Urological Association and the Society of Abdominal Radiology (Rosenkrantz, et al., 2016) state: "Non-imaging markers (i.e., PSA-based measures as well as PCA3) are likely useful in further selecting patients with a negative or low-suspicion MRI (PI-RADS
score of 1 or 2, respectively) that may deserve a systematic biopsy despite the MRI results. However, targeted biopsy remains warranted for intermediate or high suspicion MRI lesions despite results from these ancillary markers given the consistently observed strong independent effect of the MRI suspicion score on cancer detection in multivariate models. Further investigation is warranted to identify which of these markers best complements MRI findings in the repeat biopsy setting."

**BioSpeciFx**

BioSpeciFx is an individualized molecular tumor profiling of a panel of tumor markers to establish a personalized molecular profile of a tumor to recommend treatment options. It is often ordered in combination with an * invitro chemosensitivity/chemoresistance assay or ChemoFx. See CPB 245 - Tumor Chemosensitivity Assays and CPB 758 - Tumor Chemoresistance Assays. The combination of molecular profiling and * invitro drug response marker testing is sometimes referred to as comprehensive tumor profiling.

**HeproDx-TM**

mRNA expression testing for hepatocellular carcinoma (HCC) (eg, HeproDx-TM) purportedly incorporates levels of 161 genes, fresh hepatocellular carcinoma tumor tissue, AFP level and an algorithm to report a risk classifier related to HCC recurrence and metastasis.

**NETest**

NETest is a multianalyte algorithm PCR-based gene blood test that measures 51 neuroendocrine tumor specific gene transcripts in combination with molecular biomarkers which purportedly allows monitoring of neuroendocrine tumor gene activity levels.

Pęczkowska, et al. (2017) evaluated whether NETEST has clinical utility as a diagnostic and prognostic marker. The investigators conducted a prospective cohort study. Subjects included well-
differentiated arangangliomas and pheochromocytomas (PPGLs)
(n = 32), metastatic (n = 4); SDHx mutation (n = 25); 12
biochemically active, lanreotide treated (n = 4). Age- and gender-
matched controls and GEP-NETs were compared. PPGL were
NETest positive (100%). All exhibited higher scores than controls
(55 ± 5% vs 8 ± 1%, P = 0.0001), similar to GEP-NETs (47 ± 5%).
ROC analysis area under curve was 0.98 for differentiating
PPGLs/controls (cut-off for normal: 26.7%). Mutation status was
not directly linked to NETest. Genetic and molecular clustering
was associated (P < 0.04) with NETest scores. Metastatic (80 ± 9%)
and multicentric (64 ± 9%) disease had significantly (P < 0.04)
higher scores than localized disease (43 ± 7%). Progressive
disease (PD) had the highest scores (86 ± 2%) vs stable (SD, 41 ±
2%) (P < 0.0001). The area under the curve for PD from SD was
0.93 (cut-off for PD: 53%). Proliferation, epigenetic and
somatostatin receptor gene expression was elevated (P < 0.03) in
PD. Metabolic gene expression was decreased in SDHx mutations.
Repeat NETest measurements defined clinical status in the 9
patients (6 SD and 3 PD). Amine measurement was non-
informative. Multivariate analysis identified NETest >53% as an
independent prognostic factor.

Pavel, et al. (2016) assessed the NETest as a predictive and
prognostic marker of progression of gastroenteropancreatic
neuroendocrine tumors. GEP-NETs (n = 34) followed for a median
4 years (2.2-5.4) were evaluated. WHO tumor grade/stage grade
1: n = 17, grade 2: n = 14, grade 3: n = 1 (for 2, no grade was
available); 31 (91%) were stage IV. Baseline and longitudinal
imaging and blood biomarkers were available in all, and
progression was defined per standard clinical protocols (RECIST
1.0). The NETest was measured by quantitative PCR of blood and
multianalyte algorithmic analysis (disease activity scaled 0-100%
with low <40% and high activity risk cutoffs >80%); chromogranin
A (CgA) was measured by radioimmunoassay (normal <150 µg/l);
progression-free survival (PFS) was analyzed by Cox proportional-
hazard regression and Kaplan-Meier analysis. At baseline, 100%
were NETest positive, and CgA was elevated in 50%. The only
baseline variable (Cox modeling) associated with PFS was NETest
(hazard ratio = 1.022, 95% confidence interval = 1.005-1.04; p <
0.012). Using Kaplan-Meier analyses, the baseline NETest (>80%)
was significantly associated ($p = 0.01$) with disease progression (median PFS 0.68 vs. 2.78 years with <40% levels). The NETest was more informative (96%) than CgA changes (<25%) in consistently predicting disease alterations (40%, $p < 2 \times 10^{-5}$, $\chi^2 = 18$). The NETest had an earlier time point change than imaging ($1.02 \pm 0.15$ years). Baseline NETest levels >40% in stable disease were 100% prognostic of disease progression versus CgA ($\chi^2 = 5$, $p < 0.03$). Baseline NETest values <40% accurately (100%) predicted stability over 5 years ($p = 0.05$, $\chi^2 = 3.8$ vs. CgA).

Bodei, et al. (2016) assessed the accuracy of circulating NET transcripts as a measure of PRRT efficacy, and moreover to identify prognostic gene clusters in pretreatment blood that could be interpolated with relevant clinical features in order to define a biological index for the tumor and a predictive quotient for PRRT efficacy. NET patients ($n = 54$), M: F 37:17, median age 66, bronchial: $n = 13$, GEP-NET: $n = 35$, CUP: $n = 6$ were treated with ($^{177}$Lu-based)-PRRT (cumulative activity: 6.5-27.8 GBq, median 18.5). At baseline: 47/54 low-grade (G1/G2; bronchial typical/atypical), 31/49 (18)FDG positive and 39/54 progressive. Disease status was assessed by RECIST1.1. Transcripts were measured by real-time quantitative reverse transcription PCR (qRT-PCR) and multianalyte algorithmic analysis (NETest); CgA by enzyme-linked immunosorbent assay (ELISA). Gene cluster (GC) derivations: regulatory network, protein:protein interactome analyses. The disease control rate was 72%. Median PFS was not achieved (follow-up: 1-33 months, median: 16). Only grading was associated with response ($p < 0.01$). At baseline, 94% of patients were NETest-positive, while CgA was elevated in 59%. NETest accurately (89%, $\chi^2(2) = 27.4$; $p = 1.2 \times 10^{-7}$) correlated with treatment response, while CgA was 24% accurate. Gene cluster expression (growth-factor signalome and metabolome) had an AUC of $0.74 \pm 0.08$ (z-statistic = 2.92, $p < 0.004$) for predicting response (76% accuracy). Combination with grading reached an AUC: $0.90 \pm 0.07$, irrespective of tumor origin. Circulating transcripts correlated accurately (94%) with PRRT responders (SD+PR+CR; 97%) vs. non-responders (91%).

Modlin I, et al. (2016) examined whether a blood-based
multianalyte neuroendocrine gene transcript assay (NETest) would define tumor cytoreduction and therapeutic efficacy. A total of 35 GEP-NETs in 2 groups were evaluated. I: after surgery (R0, n = 15; residual, n = 12); II: nonsurgery (n = 8: embolization with gel-foam alone [bland: n = 3]), transarterial chemoembolization (n = 2), and radiofrequency embolization (n = 3). Measurement (quantitative real-time-polymerase chain reaction) and chromogranin A (CgA; enzyme-linked immunosorbent assay) were undertaken preoperatively and 1 month after treatment. NETest score was increased in 35 (100%) preoperatively; 14 (40%) had increased CgA ($\chi^2 = 30$, $P < 2 \times 10^{-8}$). Resection reduced NETest from $80 \pm 5\%$ to $29\% \pm 5$, ($P < .0001$). CgA decrease was insignificant ($14.3 \pm 1.6$ U/L to $12.2 \pm 1.7$ U/L). NETest decreases correlated with diminished tumor volume ($R^2 = 0.29$, $P = .03$). Cytoreduction significantly reduced NETest from $82 \pm 3\%$ to $41\% \pm 6$, ($P < .0001$). CgA was not decreased ($21.4 \pm 5.5$ U/L to $18.4 \pm 10.1$ U/L). Four (36%) of 11 R0s with increased NETest at 1 month developed positive imaging (sensitivity 100%, specificity 20%). One hundred percent (ablated group) were transcript- and image-positive.

Modlin, et al. (2015) reported the sensitivity and selectivity of the NETest to detect tumors with reference to other benign and malignant gastrointestinal diseases. A total of 179 cases (gastrointestinal tumors: n=81; pancreatic disease: n=98) were prospectively collected and assessed using the NETest or chromogranin A (CgA) to determine metrics for detecting small intestinal and pancreatic NETs. For intestinal carcinoids, the accuracy of the NETest was 93% (all NETs positive and 3 (12%) colorectal tumors were positive). CgA was positive in 80%, but 29% (n=7) of colorectal cancers were CgA positive. For pancreatic disease, the NETest accuracy was 94% (96% NETs positive, 2 (6%) of intraductal papillary mucinous neoplasms (IPMNs) were positive). The accuracy of CgA was 56% (29% of pancreatic NETs were CgA positive). Overall, the NETest was significantly more sensitive than CgA for the detection of small intestinal (area under the curve 0.98 vs. 0.75 $P<0.0001$) and pancreatic NETs (0.94 vs. 0.52, $P<0.0001$). NETest scores were elevated ($P<0.05$) in extensive disease and were more accurate (76-80%) than CgA levels (20-32%). The metrics of the multianalyte NETest met the
performance criteria proposed by the National Institutes of Health for biomarkers, whereas CgA measurement did not.

Modlin, et al. (2014) evaluated a PCR-based 51 transcript signature (NETest) and compared it to chromogranin A (CgA), pancreastatin (PST) and neurokinin A (NKA). The multigene signature was evaluated in two groups: i) a validation set of 40 NETs and controls and ii) a prospectively collected group of NETs (n=41, 61% small intestinal, 50% metastatic, 44% currently treated and 41 age-sex matched controls). Samples were analyzed by a two-step PCR (51 marker genes) protocol and ELISAs for CgA, PST and NKA. Sensitivity comparisons included \( \chi^2 \), non-parametric measurements, ROC curves and predictive feature importance (PFAI) analyses. NETest identified 38 of 41 NETs. Performance metrics were: sensitivity 92.8%, specificity 92.8%, positive predictive value 92.8% and negative predictive value 92.8%. Single analyte ELISA metrics were: CgA 76, 59, 65, and 71%; PST 63, 56, 59, and 61% and NKA 39, 93, 84, and 60%. The AUCs (ROC analysis) were: NETest: 0.96±0.025, CgA: 0.67±0.06, PST 0.56±0.06, NKA: 0.66±0.06. NETest significantly outperformed single analyte tests (area differences: 0.284-0.403, Z-statistic 4.85-5.9, P<0.0001). PFAI analysis determined NETest had most value (69%) in diagnosis (CgA (13%), PST (9%), and NKA (9%)). Test data were consistent with the validation set (NETest >95% sensitivity and specificity, AUC =0.98 vs single analytes: 59-67% sensitivity, AUCs: 0.58-0.63).

Chen, et al. (2017) commented that NETEST and other novel biomarkers are promising biomarkers in gastroenteropancreatic neuroendocrine tumors with potential clinical benefit, but further research is needed before their clinical application.

**Pulmotype**

A multiantibody immunohistochemistry (IHC) assay (eg, Pulmotype) purportedly aids in the differentiation of squamous and adenocarcinoma histology for NSCLC. The assay uses tissue from a lung cancer biopsy to measure five biomarkers: cytokeratin 5/6 (CK5/6), mucin-1 (MUC-1), tripartite motif containing protein 29 (TRIM 29), carcinoembryonic antigen-related cell adhesion
molecule (CEACAM) and SLC7A5. The results of these measurements are applied to an algorithm, resulting in a class assignment.

NexCourse IHC4

NexCourse IHC4 by AQUA Technology is a test proposed to determine the risk of breast cancer recurrence by analyzing protein expression of estrogen receptor (ER)/progesterone receptor (PR), HER2 and Ki67. Determination of ER/PR is performed routinely on all individuals with invasive breast cancer using immunohistochemistry (IHC) to select those individuals who are most likely to respond to hormone therapy.

EndoPredict

EndoPredict is a multi-gene assay that predicts the likelihood of women with estrogen receptor (ER)-positive and human epidermal growth factor receptor 2 (HER2)-negative breast cancer developing metastases within 10 years of the initial diagnosis (NICE, 2015). The test combines measurements of gene expression (the EndoPredict [EP] score) with nodal status and tumor size to generate a comprehensive risk score (the EPclin score) which is used to identify tumor types that will not benefit from chemotherapy.

Guidelines from the American Society for Clinical Oncology state that, "If a patient has ER/PgR-positive, HER2-negative (node-negative) breast cancer, the clinician may use the 12-gene risk score (EndoPredict; Sividon Diagnostics, K"oln, Germany) to guide decisions on adjuvant systemic chemotherapy." This is a moderate strength recommendation based upon intermediate-quality evidence. The guidelines recommend against the use of the 12-gene score to guide decisions on adjuvant systemic chemotherapy in ER/PgR-positive, HER2-negative (node-positive breast cancer. The guidelines also recommend against the use of Endopredict in patients with HER2-positive breast cancer or TN breast cancer. The guidelines recommended against the use of EndoPredict to guide decisions on extended endocrine therapy for patients with ER/PgR-positive, HER-2 negative (node-negative)
breast cancer who have had 5 years of endocrine therapy without evidence of recurrence.

An assessment by the National Institute for Health and Care Excellence (NICE, 2015) found that the published clinical evidence comes from 3 analytical validation and 5 clinical validation studies in which the test was generally shown to be reproducible and to have prognostic power. In 1 impact evaluation study, EndoPredict results were reported to change treatment decisions. A cost-effectiveness analysis found that using EndoPredict in combination with non-UK clinical guidelines was less costly and more effective than clinical guideline risk stratification alone.

Cancer Care Ontario Guidelines (Chang, et al., 2016) state: "Although no assay represents a gold standard, Oncotype DX is supported by the widest range of evidence for prognosis and prediction of chemotherapy benefit, while both Prosigna and EndoPredict have evidence-based validity in providing some of the same or similar clinical information."

**Immunohistochemistry 4 (IHC4)**

IHC4 measures the levels of 4 key proteins (ER, PR, HER2 and Ki-67) in addition to classical clinical and pathological variables (for example, age, nodal status, tumour size and grade) and calculates a risk score for distant recurrence using an algorithm (NICE, 2013). Quantitative assessments of ER, PR, and Ki-67 are needed for the IHC4 test. An online calculator for IHC4 is in development. The test uses formalin-fixed paraffin-embedded samples.

Guidelines from the American Society for Clinical Oncology (2016) state: "If a patient has ER/PgR-positive, HER2-negative (node-positive or node-negative) breast cancer, the clinician should not use immunohistochemistry 4 (IHC4) to guide decisions on adjuvant systemic chemotherapy." This is a moderate-strength recommendation based upon intermediate-quality evidence. The ASCO guidelines recommend the use of IHC4 to guide decisions on adjuvant systemic therapy for patients with HER2-positive breast cancer or TN breast cancer. The guidelines also recommended
against the use of IHC4 to guide decisions on extended endocrine therapy for patients with ER/PgR-positive, HER-2 negative (node-negative) breast cancer who have had 5 years of endocrine therapy without evidence of recurrence.

_Tumor Infiltrating Lymphocytes_

Guidelines from the American Society for Clinical Oncology (2016) state: "If a patient has ER/PgR-positive, HER2-negative (node-positive or node-negative) breast cancer, the clinician should not use tumor-infiltrating lymphocytes to guide decisions on adjuvant systemic therapy." This is a strong recommendation based upon insufficient evidence. The guidelines recommend against the use of tumor-infiltrating lymphocytes to guide decisions on adjuvant systemic therapy in patients with HER2-positive breast cancer or TN breast cancer.

_Other Markers to Guide Adjuvant Therapy in Breast Cancer_

Oncology (2016) recommends against CEP17 duplication, TIMP-1, FOXP3 and microtubule-associated protein Tau mRNA expression or mRNA expression by IHC to guide adjuvant chemotherapy selection. The ASCO guidelines also recommend against CYP2D6 polymorphisms and p27 to guide endocrine therapy selection.

_Active Surveillance of Prostate Cancers in Men with "Favorable" Intermediate-Risk Disease_

Mulcahy (2016) stated that the NCCN is the first major organization in the U.S. that has broadened the scope of prostate cancers that qualify for active surveillance to include men with "favorable" intermediate-risk disease. Active surveillance includes ongoing disease monitoring (with PSA testing, biopsies, and imaging), but does not include definitive treatment, such as surgery or radiation, or related harms, such as erectile dysfunction and incontinence. Previously, the NCCN and other organizations (e.g., the American Urological Association [AUA]) have recommended active surveillance only for very-low-risk and low-risk prostate cancers. However, the NCCN is now
recommending active surveillance for intermediate-risk prostate cancer with a Gleason score of 7 (3+4), which is considered favorable (the grade 3 prostate cancer is predominant accounting for at least 50% of the biopsied tissue, and the grade 4 is secondary accounting for at least 5% but less than 50%). Favorable intermediate risk also requires that less than 50% of a patient's biopsy cores are positive, and that he has no more than 1 NCCN intermediate-risk factor. Those risk factors include a tumor stage of T2b to T2c and a PSA value of 10 to 20 ng/ml. Moreover, the new NCCN recommendation does not include active surveillance for "unfavorable" intermediate-risk cancer, such as that with a Gleason score of 7 (4+3).

Dr. James Mohler of the NCCN Prostate Cancer Panel noted that the use of active surveillance in select men with intermediate-risk prostate cancer has been underway for years at major academic centers. However, the new NCCN recommendation is a "little more inclusive than what many urologists have used for selecting these [favorable intermediate-risk] patients. Specifically, it is "more liberal" in terms of the percentage of positive biopsies it allows and, thus, will allow an even higher percentage of intermediate-risk patients to be monitored with active surveillance". The recommendation is based on a radiation oncology study in which 1,024 patients with intermediate-risk prostate cancer underwent radiotherapy with or without androgen-deprivation therapy (Zumsteg et al, 2013). In that study, the authors analyzed recurrence-free survival and distant metastases outcomes, and concluded that intermediate-risk disease is "heterogenous" with "favorable and unfavorable subsets" -- groupings that the NCCN is now using. The idea that active surveillance should be used in favorable intermediate-risk disease was endorsed by Dr. Ann Raldow and her colleagues of the Harvard Radiation Oncology Program. In their observational study, Raldow et al (2015) compared 3,972 men with low-risk prostate cancer with 1,608 men with favorable intermediate-risk prostate cancer, all of whom were treated with brachytherapy from 1997 to 2013. These investigators found that rates of prostate-cancer-specific mortality and all-cause mortality were similar in the 2 groups, and concluded that these findings provided "evidence to support active surveillance as an initial
approach for men with favorable intermediate-risk prostate cancer".

Dr. Mohler stated that the NCCN Prostate Cancer Panel has been advocating active-surveillance for some time. In 2010, the NCCN was the first organization to recommend active surveillance as the sole initial therapy for many men. The use of active surveillance has increased in recent years in the U.S.; estimates range from a high of 50% of men with low-risk prostate cancer in Michigan, where an insurance-industry-funded awareness program is underway, to a low of 8% nationally. Medscape Medical News reported that another estimate of the prevalence of the practice came from CaPSURE, a prostate cancer registry that has been collecting data on men managed at 47 clinical, primarily community-based, sites. An analysis of the data collected from 2008 to 2013 showed that the primary treatment of 38.4% of men with low-risk tumors was watchful waiting or active surveillance. Furthermore, Dr. Stacy Loeb of the New York University stated that the U.S. has lagged behind certain European countries in the adoption of active surveillance. As of 2013, the use of active surveillance in Sweden was 78% for men with very-low-risk disease and 59% for men with low-risk disease.

CDX2 as a Prognostic Biomarker in Colon Cancer:

Dalerbra and colleagues (2016) stated that the identification of high-risk stage II colon cancers is key to the selection of patients who require adjuvant treatment after surgery. Microarray-based multigene-expression signatures derived from stem cells and progenitor cells hold promise, but they are difficult to use in clinical practice. These investigators used a new bioinformatics approach to search for biomarkers of colon epithelial differentiation across gene-expression arrays and then ranked candidate genes according to the availability of clinical-grade diagnostic assays. With the use of subgroup analysis involving independent and retrospective cohorts of patients with stage II or stage III colon cancer, the top candidate gene was tested for its association with DFS and a benefit from adjuvant chemotherapy. The transcription factor CDX2 ranked first in the screening test. A
group of 87 of 2,115 tumor samples (4.1 \%) lacked CDX2 expression. In the discovery data set, which included 466 patients, the rate of 5-year DFS was lower among the 32 patients (6.9 \%) with CDX2-negative colon cancers than among the 434 (93.1 \%) with CDX2-positive colon cancers (HR for disease recurrence, 3.44; 95 % CI: 1.60 to 7.38; p = 0.002). In the validation data set, which included 314 patients, the rate of 5-year DFS was lower among the 38 patients (12.1 \%) with CDX2 protein-negative colon cancers than among the 276 (87.9 \%) with CDX2 protein-positive colon cancers (HR, 2.42; 95 % CI: 1.36 to 4.29; p = 0.003). In both these groups, these findings were independent of the patient's age, sex, and tumor stage and grade. Among patients with stage II cancer, the difference in 5-year DFS was significant both in the discovery data set (49 \% among 15 patients with CDX2-negative tumors versus 87 \% among 191 patients with CDX2-positive tumors, p = 0.003) and in the validation data set (51 \% among 15 patients with CDX2-negative tumors versus 80 \% among 106 patients with CDX2-positive tumors, p = 0.004). In a pooled database of all patient cohorts, the rate of 5-year DFS was higher among 23 patients with stage II CDX2-negative tumors who were treated with adjuvant chemotherapy than among 25 who were not treated with adjuvant chemotherapy (91 \% versus 56 \%, p = 0.006). the authors concluded that lack of CDX2 expression identified a subgroup of patients with high-risk stage II colon cancer who appeared to benefit from adjuvant chemotherapy. They stated that given the exploratory and retrospective design of the study, these findings need to be validated; they advocated these results to be confirmed within the framework of randomized, clinical trials, in conjunction with genomic DNA sequencing studies.

**PDGFRB Testing:**

The National Comprehensive Cancer Network's Biomarkers Compendium (2016) recommends the following for PDGFRB testing:

**Myelodysplastic Syndromes (MDS):** Helpful in some clinical situations: Evaluate CMML patients for 5q31-33 translocations and/or PDGFR beta gene rearrangements. (Category of Evidence:
Non-Melanoma Skin Cancers - Dermatofibrosarcoma Protuberans (DFSP): Tumors lacking the t(17;22) translocation may not respond to imatinib. Molecular analysis of a tumor using cytogenetics may be useful prior to the institution of imatinib therapy. (Category of Evidence: 2A).

Guardant360

The Guardant360 panel analyzes cell-free circulating tumor DNA (liquid biopsy) for 73 genes associated with a wide variety of solid tumors.

Noting that data on the influence of hybrid capture (HC)-based NGS on treatment are limited, Rozenblum, et al. (2017) investigated its impact on treatment decisions and clinical outcomes in a series of patients at a cancer center. This retrospective study included patients with advanced lung cancer on whom HC-based NGS with broad gene panels was performed between November 2011 and October 2015. HC-based NGS was performed upon the recommendation of the treating physician, mostly on the basis of young age and smoking history. The results of standard molecular testing for EGFR mutations and ALK rearrangements were negative before HC-based NGS in 80.2% (81 of 101) and 70.3% (71 of 101) of the patients, respectively. Upfront HC-based NGS was performed on 15 patients because of very little biopsy material. HC-based NGS was performed off-site on tumor samples with FoundationOne (Foundation Medicine, Inc., Cambridge, MA) (n = 82) or on blood samples using a liquid biopsy approach with Guardant360 if the tissue sample had been exhausted (n= 18). The study focused on gene analyses (GAs) with potential clinical relevance. Initial analysis (level 1) included GAs associated with U.S. Food and Drug Administration–approved anticancer therapies (including off-label drugs) for all cancer types. A subsequent analysis (level 2) included GAs with appropriate evidence-based targeted agents with antidriver activity in lung cancer, as recommended by the National Comprehensive Cancer Network (NCCN) guidelines for NSCLC. GAs associated with investigational treatments were not included
in the current analysis. Demographic and clinicopathologic characteristics, treatments, and outcome data were collected. A total of 101 patients were included (median age 63 years [53% females, 45% never-smokers, and 85% with adenocarcinoma]). HC-based NGS was performed upfront and after EGFR/ALK testing yielded negative or inconclusive results in 15% and 85% of patients, respectively. In 51.5% of patients, HC-based NGS was performed before first-line therapy, and in 48.5%, it was performed after treatment failure. HC-based NGS identified clinically actionable genomic alterations in 50% of patients, most frequently in EGFR (18%), Ret proto-oncogene (RET) (9%), ALK (8%), Mesenchymal-epithelial transition factor (MET) receptor tyrosine kinase gene (6%), and erb-b2 receptor tyrosine kinase 2 gene (ERBB2) (5%). In 15 patients, it identified EGFR/ALK aberrations after negative results of prior standard testing. Treatment strategy was changed for 43 patients (42.6%). The overall response rate in these patients was 65% (complete response 14.7%, partial response 50%). Median survival was not reached. Immunotherapy was administered in 33 patients, mostly without an actionable driver, with a presenting disease control rate of 32%, and with an association with tumor mutation burden. The authors noted a number of limitations of this study, including its retrospective nature, its small sample size, and its being a single-center study. In addition, the high percentage of never-smokers, the preponderance of female patients, and the relatively young median age of the patient group represented a selection bias with a high pretest probability for the existence of driver mutation. The authors noted that the results of large prospective trials such as the UK National Lung Matrix Trial and the National Cancer Institute’s Molecular Analysis for Therapy Choice Program are thus eagerly anticipated.

Noting that there is a paucity of data on the concordance between plasma cell-free circulating tumor DNA (ctDNA) and tissue-based genomic testing, Villaflor, et al. (2016) reported on a descriptive study of subjects with NSCLC undergoing analysis of ctDNA using Guardant360 next-generation sequencing assay at a single institution. The authors stated that this study is the first clinic-based series of NSCLC patients assessing outcomes of targeted therapies using a commercially available ctDNA assay. Of
the 90 patients submitted for ctDNA analysis as part of clinical care, 68 had provided informed consent for inclusion in this study. Thirty-eight samples from the 68 subjects were tested using the 54-gene ctDNA panel while the remaining 31 samples were analyzed on the 68-gene ctDNA panel. Of note, the 54-gene panel did not include ALK, RET or ROS1 fusions. Tissue-based testing was performed on 44 subjects using 9 different testing platforms. Demographic, clinicopathologic information and results from tissue and plasma-based genomic testing were reviewed for each subject. The majority of patients had a diagnosis of lung adenocarcinoma (n = 55, 81%), with the remainder lung squamous cell carcinoma (n = 12, 17.7%) and other lung cancers (n = 1, 1.3%). Over 80% of patients had detectable ctDNA. Thirty-one patients had matched tissue and blood samples; the reason for lack of tissue results for the remaining 37 patients was not routinely documented. In cases with detectable ctDNA and completed tissue analysis, an EGFR activating was found in both tissue and blood in 5 paired samples, and in tissue only in 2 samples (71% concordance). The time between biopsy and blood draw ranged from 0 days to 7 years, with an average of 8.8 months and median of 1.4 years between biopsy and blood draw. The investigators found no correlation between concordance and timing of blood draw versus tissue biopsy. A total of 9 subjects with paired tissue and blood samples had an EGFR driver mutation identified in plasma and tissue (n = 5), plasma only (n = 1) or tissue only (n = 3). Eight of these individuals were treated with erlotinib or afatinib at first or second line. Two patients were still responding to therapy at the time of data analysis. Of the 6 remaining patients, the median progression-free survival was 11.5 months (range 7.5 months–29 months; 95% CI–5.7–28.7). The investigators stated that these data suggest that biopsy-free ctDNA analysis is a viable first choice when the diagnostic tissue biopsy is insufficient for genotyping or at the time of progression when a repeated invasive tissue biopsy is not possible/prefereed. The authors noted however, that the numbers in this series are modest and further research in larger prospective cohorts is needed.

Thompson, et al. (2016) evaluated the feasibility of using cell-free circulating tumor DNA (ctDNA) NGS as a complement or
alternative to tissue NGS in a single-center observational study. A total of 112 plasma samples obtained from a consecutive study of 102 prospectively enrolled patients with advanced NSCLC were subjected to ultra-deep sequencing of 68 or 70 genes and matched with tissue samples, when possible. The investigators detected 275 alterations in 45 genes, and at least one alteration in the ctDNA for 86 of 102 patients (84%), with EGFR variants being most common. ctDNA NGS detected 50 driver and 12 resistance mutations, and mutations in 22 additional genes for which experimental therapies, including clinical trials, are available. Although ctDNA NGS was completed for 102 consecutive patients, tissue sequencing was only successful for 50 patients (49%). The overall concordance for all variants covered and detected by both platforms was 60%. When wild-type calls, that is, genes for which no variants were detected, are considered, the overall concordance was 97.5%. Actionable EGFR mutations were detected in 24 tissue and 19 ctDNA samples, yielding concordance of 79%, with a shorter time interval between tissue and blood collection associated with increased concordance \((P = 0.038)\). ctDNA sequencing identified eight patients harboring a resistance mutation who developed progressive disease while on targeted therapy, and for whom tissue sequencing was not possible.

Schwaederle, et al. (2016) extracted plasma from 171 patients with a variety of cancers and analyzed the plasma for ctDNA (54 genes and copy number variants (CNVs) in three genes (EGFR, ERBB2 and MET)). The most represented cancers were lung (23%), breast (23%), and glioblastoma (19%). Ninety-nine patients (58%) had at least one detectable alteration, where actionability was defined as an alteration that was either the direct target or a pathway component that could be targeted by at least one FDA approved or investigational drug in a clinical trial. The most frequent alterations were TP53 (29.8%), followed by EGFR (17.5%), MET (10.5%), PIK3CA (7%), and NOTCH1 (5.8%). In contrast, of 222 healthy volunteers, only one had an aberration (TP53). Ninety patients with non-brain tumors had a discernible aberration (65% of 138 patients; in 70% of non-brain tumor patients with an alteration, the anomaly was potentially actionable). Nine of 33 patients (27%) with glioblastoma had an
alteration (6/33 (18%) potentially actionable). Overall, sixty-nine patients had potentially actionable alterations (40% of total; 69.7% of patients (69/99) with alterations); 68 patients (40% of total; 69% of patients with alterations), by an FDA-approved drug. In summary, 65% of diverse cancers (as well as 27% of glioblastomas) had detectable ctDNA aberration(s), with the majority theoretically actionable by an approved agent. The authors noted a number of study limitations. First, this study included a limited number of patients in each histology. Second, clinical annotation was not available since the database was de-identified. Third, the definition of "actionable" and the level of evidence needed for such a determination is a matter of debate and in constant evolution. Fourth, the use of tissue-based next generation sequencing as a comparison to establish clinical utility was not accessible for this group of de-identified patients. Finally, whether or not the patients would have responded to these drugs could not be addressed in this study, and will require further investigation.

Liang, et al. (2016) performed a retrospective chart review of 100 patients with stage 4 or high-risk stage 3 breast cancer. Of the 100 patients included in this study, 29 had a tissue analysis done during the course of treatment. Only the specific genomic alterations tested in both the cell-free DNA and tissue DNA were included in this analysis. Of the 29 patients with tissue analysis, six had no evidence of disease at the time of cell-free DNA analysis and were excluded from the comparative analysis of genomic alterations found between cell-free DNA and tissue DNA. Fifty-five single nucleotide variants (SNVs) and 4 copy number variants (CNVs) were evaluated for both cell-free DNA and tissue DNA from the 23 remaining patients. The degree of agreement between genomic alterations found in tumor DNA (tDNA) and cfDNA was determined by Cohen's Kappa. Clinical disease progression was compared to mutant allele frequency using a two-sided Fisher's exact test. The presence of mutations and mutant allele frequency was correlated with progression-free survival (PFS) using a Cox proportional hazards model and a log-rank test. The most commonly found genomic alterations were mutations in TP53 and PIK3CA, and amplification of EGFR and ERBB2. PIK3CA mutation and ERBB2 amplification demonstrated
robust agreement between tDNA and cfDNA (Cohen’s kappa = 0.64 and 0.77, respectively). TP53 mutation and EGFR amplification demonstrated poor agreement between tDNA and cfDNA (Cohen’s kappa = 0.18 and 0.33, respectively). The directional changes of TP53 and PIK3CA mutant allele frequency were closely associated with response to therapy (p = 0.002). The investigators stated that the presence of TP53 mutation (p = 0.0004) and PIK3CA mutant allele frequency [p = 0.01, HR 1.074 (95 % CI 1.018-1.134)] was excellent predictors of PFS. The investigators concluded that identification of selected cancer-specific genomic alterations from cfDNA may be a noninvasive way to monitor disease progression, predict PFS, and offer targeted therapy. The investigators noted that this study is limited by its small sample size and the inherent nature of retrospective data collection of existing genomic information.

CancerIntercept

CancerIntercept (Pathway Genomics) is a liquid biopsy intended for use as a non-invasive screening test designed for early cancer detection and monitoring. Cell-free DNA (cfDNA) in the blood is tested for the presence of circulating tumor DNA (ctDNA) by screening for specific cancer-associated mutations using polymerase chain reaction (PCR) to amplify both the mutant and wild type DNA, followed by a “specific enrichment of the mutant and simultaneous removal of the wild type DNA by using a proprietary technology,” after which the “mutant DNA is sequenced on Illumina’s next-generation sequencing platform.” The tests analyze the presence of 96 frequently occurring DNA mutation hot spots in nine cancer driver genes (BRAF, CTNNB1, EGFR, FOXL2, GNAS, KRAS, NRAS, PIK3CA and TP53) that, when mutated, can cause cancer or contribute to cancer progression. These mutations are commonly associated with lung, breast, ovarian, colorectal cancers and melanoma, and may occur less frequently in other cancer types (such as pancreatic, head and neck, thyroid, gastric and prostate cancers). Clinical trials are ongoing to assess the correlation of liquid biopsy results with the actual presence or absence of these mutations in the tumor itself.

The test is offered for two general indications: CancerIntercept
Detect is a liquid biopsy designed to detect tumor DNA in high-risk individuals; CancerIntercept Monitor is intended to monitor patients with active or previously diagnosed cancer.

There is a lack of adequate clinical validation to justify CancerIntercept Detect's recommended use in screening high-risk patients for cancer. There is a lack of clinical trial evidence showing that CancerIntercept Detect results in earlier diagnosis or decreases mortality from cancer.

Clinical trials are also examining the prognostic value of various mutations screened for by the CancerIntercept Monitor test in terms of recurrence, survival, and response to treatment.

*Circulating Cell-Free Nucleic Acids in Colorectal Cancer*

Toth and colleagues (2016) stated that screening methods for the most frequent diagnosed malignant tumor, CRC, have limitations. Total circulating cell-free DNA (cfDNA) analysis came into focus as a potential screening test for CRC. Detection of epigenetic and genetic alterations of cfDNA as DNA methylation or DNA mutations and related ribonucleic acids may improve cancer detection based on unique, CRC-specific patterns. These investigators summarized the CRC-specific nucleic acid biomarkers measured in peripheral blood and their potential as screening markers. Detection of DNA mutation has inadequate sensitivity; however, methylated DNA can be established with higher sensitivity from CRC plasma samples. The ribonucleic acid based miRNA studies represented higher sensitivity for CRC as compared with mRNA studies. Recently, isolation of cfDNA has become automated, highly reproducible and a high throughput method. The authors concluded that with automated possible diagnostic tools, a new approach may be available for CRC screening as liquid biopsy.

Spindler (2017) noted that circulating DNA can be used to measure cfDNA and for detection and quantification of tumor-specific genetic alterations in the peripheral blood, and the broad clinical potential of circulating DNA has attracted increasing focus over the past decade. Concentrations of circulating DNA are high
in metastatic CRC, and the total levels of cfDNA have been reported to hold strong prognostic value. Colorectal tumors are characterized by a high frequency of well known, clinically relevant genetic alteration, which is readily detected in the cfDNA and holds potential for tailoring of palliative therapy and for monitoring during treatment. These investigators reviewed the current literature that has specifically reported data on the potential utility of cfDNA and on tumor-specific mutations in metastatic CRC (mCRC). Methodological, biological and clinical aspects were discussed based on the most recent development in this specific setting, and eligible studies were identified by systematic literature searched from PubMed and Embase in addition to conference papers and communications. The literature regarding cfDNA in CRC is broad and heterogeneous concerning aims, nomenclature, methods, cohorts and clinical end-points and consequently difficult to include in a single systematic search. However, the available data underline a strong clinical value of measuring both total cfDNA levels and tumor-specific mutations in the plasma of patients with mCRC, pre- and during systemic therapy. The authors concluded that this paper had gathered the most recent literature on several aspects of cfDNA in mCRC, including methodological, biological and clinical aspects, and discussed the large clinical potential in this specific setting, which needs to be validated in carefully designed prospective studies in statistically relevant cohorts.

HMGB1 and RAGE in Cutaneous Malignancy

Tesarova and associates (2016) noted that activation of the receptor for advanced glycation end-products (RAGE) due to its increased expression in cancer cells or its stimulation by multiple ligands (AGEs, high-mobility group box-1 [HMGB1], S100 proteins, etc.) may contribute to the proliferation, invasiveness of tumor cells and formation of distant metastases and also to the resistance of cancer to treatment. RAGE ligands could thus become both useful markers of disease severity and its outcome and, a potential therapeutic target. The authors concluded that better understanding of the role of RAGE activation in different types of cancer may help to define the role of ligand/RAGE antagonists as promising cancer treatment.
Nguyen and colleagues (2017) stated that inflammation and the immune system play a role in the development and progression of melanoma, basal cell carcinoma (BCC), and SCC. The pro-inflammatory and tumor-promoting effects of HMGB1 protein and RAGE have been investigated in these cutaneous malignancies. The clinical implication of these molecules is not fully described. The National Library of Medicine database was searched for articles addressing the clinical relevance of HMGB1 and RAGE in melanoma, BCC, and SCC. This systematic review included 9 articles, with 6 summarizing RAGE in cutaneous malignancies and 3 involving HMGB1. RAGE has been found to be up-regulated in SCC lesions, as well as melanoma. Levels of RAGE were highest in stage IV melanomas. Lower levels of soluble RAGE have been associated with poor OS in melanoma. Sporadic extracellular expression of HMGB1 was evident in BCC and SCC lesions, which could be released by necrotic tumor cells. HMGB1 was found to be a prognostic marker in melanoma, and HMGB1 levels were elevated in patients who were non-responders to ipilimumab treatment. The authors concluded that HMGB1 and RAGE could serve as potential prognostic markers or therapeutic targets in treating melanoma, BCC, and SCC; however, further research regarding the clinical utility of the HMGB1-RAGE axis in cutaneous malignancies is needed.

**Ki-67 in Upper Tract Urinary Carcinoma**

Ley and associates (2015) noted that upper urinary-tract urothelial carcinomas (UTUC) constitute 5% of urothelial malignancies. Prognostic biomarkers would allow lower risk surgical approaches for less aggressive UTUCs. One biomarker, Ki-67/mindbomb E3 ubiquitin protein ligase 1 (Ki-67/MIB-1), showed promise in UTUC, but there have been conflicting findings regarding its prognostic role. This systematic review and meta-analysis examined the prognostic value of Ki-67/MIB-1 in UTUC in terms of UTUC-specific mortality rate, 5-year DFS, and 5-year OS (including DSS). A systematic review of the current literature produced 654 records. A total of 13 studies consisting of 1,030 patients were finally included in the meta-analysis; HRs with 95% CI were extracted or estimated. The individual HR estimates were combined into a pooled HR using a fixed-effects
model that summed homogeneity of the individual true HRs. Patients with Ki-67/MIB-1 over-expression displayed significantly higher UTUC-specific mortality rate (pooled HR: 2.14, 95 % CI: 1.73 to 2.64; p < 0.00001), significantly reduced 5-year DFS (pooled HR: 2.27, 95 % CI: 1.79 to 2.92; p < 0.00001), and significantly reduced 5-year OS (pooled HR = 1.77; 95 % CI: 1.39 to 2.23 p < 0.00001). There was significant heterogeneity detected in the UTUC-specific mortality rate meta-analysis (I(2) = 63 %) and the 5-year DFS meta-analysis (I(2) = 65 %), but there was no significant heterogeneity detected in the 5-year OS meta-analysis (I(2) = 0%). Egger's testing showed that none of the outcomes was influenced by publication bias (p > 0.05). The authors concluded that Ki-67/MIB-1 over-expression showed promise as a prognostic biomarker for UTUC patients and required further investigation.

Fan and colleagues (2016) stated that UTUC is a relatively uncommon but aggressive disease. The Ki-67 antigen is a classic marker of cellular proliferation, but there is still controversy regarding the significance and importance of Ki-67 in tumor progression. In this study, these researchers first detected Ki-67 expression in UTUC patients by immunohistochemistry. Subsequently, they quantitatively combined the results with those from the published literature in a meta-analysis after searching several databases. Immunohistochemistry results demonstrated that patients with muscle-invasive tumors (T2-T4) had higher Ki-67 expression than those with non-muscle-invasive tumors (Tis-T1), suggesting that high Ki-67 expression may be associated with the aggressive form of UTUC. Kaplan-Meier curves showed that patients with high Ki-67 expression had significantly poorer cancer-specific survival (CSS) and DFS. Furthermore, multi-variate analysis suggested that Ki-67 expression was an independent prognostic factor for CSS (HR = 3.196) and DFS (HR = 3.517) in UTUC patients. Then, a meta-analysis of the published literature investigating Ki-67 expression and its effects on UTUC prognosis was conducted. After searching the PubMed, Medline, Embase, Cochrane Library and Scopus databases, a total of 12 articles met the eligibility criteria for this analysis. The eligible studies included a total of 1,740 patients with a mean number of 82 patients per study (range of 38 to
The combined results showed that increased Ki-67 levels were associated with poor survival and disease progression, with a pooled HR estimate of 2.081 and 2.791, respectively. In subgroup analysis, the pooled HR was statistically significant for CSS (HR = 2.276), metastasis-free survival (HR = 3.008) and DFS (HR = 6.336). The authors concluded that high Ki-67 expression was associated with poor survival in patients with UTUC, as well as a high risk of disease progression, although these findings need to be interpreted with caution. They stated that large-scale, adequately designed, prospective trials are needed to further confirm the value of Ki-67 in prognosis of UTUC patients.

Long Non-Coding RNA in Gallbladder Cancer and Non-Small Cell Lung Cancer

Ricciuti and associates (2016) stated that recent advances in tiling array and high throughput analyses revealed that at least 87.3% of the human genome is actively transcribed, though less than 3% of the human genome encodes proteins. This unexpected truth suggests that most of the transcriptome is constituted by non-coding RNA. Among them, high-resolution microarray and massively parallel sequencing analyses identified long non-coding RNAs (lncRNAs) as non-protein-coding transcripts. lncRNAs are largely poly-adenylated and greater than 200 nucleotides in length transcripts, involved in gene expression through epigenetic and transcriptional regulation, splicing, imprinting and subcellular transport. Although lncRNAs functions are largely uncharacterized, accumulating data indicate that they are involved in fundamental biological functions. Conversely, their dysregulation has increasingly been recognized to contribute to the development and progression of several human malignancies, especially lung cancer, which represents the leading cause of cancer-related deaths worldwide. These researchers conducted a comprehensive review of the published literature focusing on lncRNAs function and disruption in non-small cell lung cancer (NSCLC) biology, also highlighting their value as biomarkers and potential therapeutic targets. lncRNAs are involved in NSCLC pathogenesis, modulating fundamental cellular processes such as proliferation, cell growth, apoptosis, migration, stem cell maintenance and epithelial to mesenchymal transition, also
serving as signaling transducers, molecular decoys and scaffolds. Furthermore, lncRNAs represent very promising biomarkers in early-stage NSCLC patients and may become particularly useful in non-invasive screening protocols. lncRNAs may be used as predictive biomarkers for chemotherapy and targeted therapies sensitivity. In addition, selectively targeting oncogenic lncRNAs could provide a new therapeutic tool in treating NSCLC patients. The authors concluded that lncRNAs disruption plays a pivotal role in NSCLC development and progression. They stated that these molecules also serve as diagnostic, prognostic and predictive biomarkers; characterization of lncRNA genes and their mechanisms of action will foster development of a more comprehensive clinical approach, with the final goal to benefit patients.

Xu and colleagues (2016) noted that lung cancer ranks as the first most common cancer and the first leading cause of cancer-related death in China and worldwide. Due to the difficulty in early diagnosis and the onset of cancer metastasis, the 5-year survival rate of lung cancer remains extremely low. Long noncoding RNAs, which lacking protein-coding ability, have recently emerged as pivotal participants in biological processes, often dysregulated in a range of cancers, including lung cancer. These investigators highlighted the recent findings of lncRNAs in lung cancer pathogenesis. The authors concluded that while the understanding of lncRNAs in the onset and progression of lung cancer is still in its infancy, there is no doubt that understanding the activities of lncRNAs will certainly secure strong biomarkers and improve treatment options for lung cancer patients.

Furthermore, National Comprehensive Cancer Network’s clinical practice guidelines on “Non-small cell lung cancer” (Version 4.2017) does not mention detection of long non-coding RNAs as a management tool.

Khandelwal and colleagues (2017) noted that gallbladder cancer (GBC) is the most common and aggressive form of biliary tract carcinoma with an alarmingly low 5-year survival rate. Despite its high mortality rate, the underlying mechanisms of GBC pathogenesis are not completely understood. Recently, from a
growing volume of literature, long non-coding RNAs (lncRNAs) have emerged as key regulators of gene expression and appear to play vital roles in many human cancers. To-date, a number of lncRNAs have been implicated in GBC, but their potential roles in GBC have not been systematically examined. These investigators discussed the emerging roles of lncRNAs in GBC, and the pathways involved. Specifically, they noted that some lncRNAs show greater expression in T1 and T2 tumor stages compared to T3 and T4 tumor stages and that their dysregulation leads to alterations in cell cycle progression and can cause an increase in GBC cell proliferation or apoptosis. Furthermore, some lncRNAs control the epithelial-mesenchymal transition process, while others take part in the regulation of ERK/MAPK and Ras cancer-associated signaling pathways. These researchers also presented their potential utility in diagnosis, prognosis, and/or treatment of GBC. The authors concluded that the overall goal of this review was to stimulate interest in the role of lncRNAs in GBC, which may open new avenues in the determination of GBC pathogenesis and may lead to the development of new preventive and therapeutic strategies for GBC.

**MUC1 in Gastric Cancer**

Wang and colleagues (2016) stated that MUC1, a member of the mucin family, is expressed in tumors of various human organs and may function as an anti-adhesion molecule that inhibits cell-to-cell adhesion, inducing tumor metastasis, and served as a potential biomarker of tumor progression in early gastric cancer. However, its prognostic significance in gastric cancer is still in dispute. These researchers performed a meta-analysis to evaluate the relationship between MUC1 expression and prognosis of gastric cancer. A total of 10 eligible studies with 834 cases and 548 controls were included. MUC1 positive cases were highly positive in intestinal-type carcinomas (OR = 1.76, 95 % CI: 1.27 to 2.44, p = 0.0008 fixed-effect), higher rate of vascular invasion (OR = 1.64, 95 % CI: 1.13 to 2.39, p = 0.009 fixed-effect), and lymph node metastasis (OR = 2.10, 95 % CI: 1.20 to 3.67, p = 0.01 random-effect), as well as lower 5-year survival rate (HR = 0.27, 95 % CI: 0.11 to 0.66, p = 0.004 random-effect). However, the presence of MUC1 was not associated with gender, tumor
size, histologic differentiation, and clinical stage. The authors concluded that MUC1 is a prognostic factor in gastric cancer, which acts as a marker of poor outcome in patients with gastric cancer; further clinical studies are needed to confirm the role of MUC1 in clinical practice.

Furthermore, National Comprehensive Cancer Network’s clinical practice guidelines on “Gastric cancer” (Version 3.2016) did not mention the use of MUC1 as a biomarker.

Perceptua Bronchial Genomic Classifier (Veracyte)

Silvestri et al (2015) stated that bronchoscopy is frequently non-diagnostic in patients with pulmonary lesions suspected to be lung cancer. This often results in additional invasive testing, although many lesions are benign. These researchers sought to validate a bronchial-airway gene-expression classifier that could improve the diagnostic performance of bronchoscopy. Current or former smokers undergoing bronchoscopy for suspected lung cancer were enrolled at 28 centers in 2 multi-center prospective studies (AEGIS-1 and AEGIS-2). A gene-expression classifier was measured in epithelial cells collected from the normal-appearing main-stem bronchus to assess the probability of lung cancer. A total of 639 patients in AEGIS-1 (298 patients) and AEGIS-2 (341 patients) met the criteria for inclusion. A total of 43 % of bronchoscopic examinations were non-diagnostic for lung cancer, and invasive procedures were performed after bronchoscopy in 35 % of patients with benign lesions. In AEGIS-1, the classifier had an area under the receiver-operating-characteristic curve (AUC) of 0.78 (95 % confidence interval [CI]: 0.73 to 0.83), a sensitivity of 88 % (95 % CI: 83 to 92), and a specificity of 47 % (95 % CI: 37 to 58). In AEGIS-2, the classifier had an AUC of 0.74 (95 % CI: 0.68 to 0.80), a sensitivity of 89 % (95 % CI: 84 to 92), and a specificity of 47 % (95 % CI: 36 to 59). The combination of the classifier plus bronchoscopy had a sensitivity of 96 % (95 % CI: 93 to 98) in AEGIS-1 and 98 % (95 % CI: 96 to 99) in AEGIS-2, independent of lesion size and location. In 101 patients with an intermediate pre-test probability of cancer, the negative predictive value of the classifier was 91 % (95 % CI: 75 to 98) among patients with a non-diagnostic bronchoscopic
examination. The authors concluded that the gene-expression classifier improved the diagnostic performance of bronchoscopy for the detection of lung cancer. In intermediate-risk patients with a non-diagnostic bronchoscopic examination, a negative classifier score provided support for a more conservative diagnostic approach.

The authors noted that there are several important limitations to this study: (i) specimens from 155 patients (11%) yielded insufficient or poor-quality RNA, precluding measurement of the classifier. However, similar rates of insufficient RNA quality or quantity have been observed with other gene expression tests that have been integrated into clinical practice, and it may be possible to improve sample quality by decreasing the time between sample collection and RNA isolation. Patients who were not included in the study for this reason do not appear to differ in terms of cancer prevalence or other clinical features in comparison with the overall study population; however, it cannot be determined whether the classifier has similar performance in this group; (ii) 9% of patients were lost to follow-up, and 5% did not have a definitive diagnosis established at 12 months after bronchoscopic examination. This rate of loss to follow-up is not unexpected in an observational trial in which the subsequent evaluation after bronchoscopic examination was not mandated to occur at the study center. Although the follow-up period was limited to 12 months, it is unlikely that they missed a substantial number of cancers that would have been found with an additional year of follow-up. Although guidelines suggest 24 months of surveillance, these recommendations are based on older studies regarding solitary pulmonary nodules discovered on chest radiography (not computed tomography [CT]). The high sensitivity of CT makes it unlikely that solid nodules that are stable in the first year will have subsequent growth; this is supported by studies of lung cancer screening in which nodules that were stable for 1 year had a conversion rate to cancer of only 1 per 1,000 during year 2; (iii) the exclusion criteria in this study limit the generalizability of these findings among life-time non-smokers and smokers with a history of lung cancer. It is unclear whether a similar field of injury exists in people who have never smoked or in very light smokers who have lung cancer and
whether the field of injury persists after tumor resection; further studies are needed to evaluate these questions; (iv) these investigators considered bronchoscopy to be “diagnostic” only when the procedure yielded a lung-cancer diagnosis. There were 49 bronchoscopic examinations in which a specific benign cause was identified, but 31 of the patients received further invasive testing, including 4 patients who ultimately had lung cancer diagnosed on subsequent lung biopsy; this suggests that the concern for cancer remained elevated despite the initial benign finding on bronchoscopic examination; and (v) these researchers did not assess the accuracy of a model incorporating the classifier in combination with clinical variables. Although risk-prediction models have been developed for solitary pulmonary nodules, there are no validated models for patients undergoing diagnostic bronchoscopic examination, which includes patients with a broad range of findings, including larger lesions (i.e., greater than 3 cm), infiltrates, or lymphadenopathy. Thus, most patients are selected for bronchoscopy on the basis of the physician’s qualitative assessment of the probability of lung cancer. The authors showed that their classifier performed well in patients with an intermediate probability of cancer as assessed by a physician in a process that incorporated the available clinical risk factors.

Whitney et al (2015) stated that the gene expression profile of cytologically-normal bronchial airway epithelial cells has previously been shown to be altered in patients with lung cancer. Although bronchoscopy is often used for the diagnosis of lung cancer, its sensitivity is imperfect, especially for small and peripheral suspicious lesions. In this study, these researchers derived a gene expression classifier from bronchoscopically-obtained airway epithelial cells that detects the presence of cancer in current and former smokers undergoing bronchoscopy for suspect lung cancer and evaluated its sensitivity to detect lung cancer among patients from an independent cohort. They collected bronchial epithelial cells (BEC) from the main-stem bronchus of 299 current or former smokers (223 cancer-positive and 76 cancer-free subjects) undergoing bronchoscopy for suspected lung cancer in a prospective, multi-center study. RNA from these samples was run on gene expression microarrays for training a gene-expression classifier. A logistic regression model
was built to predict cancer status, and the finalized classifier was validated in an independent cohort from a previous study. These researchers found 232 genes whose expression levels in the bronchial airway were associated with lung cancer. They then built a classifier based on the combination of 17 cancer genes, gene expression predictors of smoking status, smoking history, and gender, plus patient age. This classifier had a ROC curve AUC of 0.78 (95% CI: 0.70 to 0.86) in patients whose bronchoscopy did not lead to a diagnosis of lung cancer (n = 134). In the validation cohort, the classifier had a similar AUC of 0.81 (95% CI: 0.73 to 0.88) in this same subgroup (n = 118). The classifier performed similarly across a range of mass sizes, cancer histologies and stages. The negative predictive value was 94% (95% CI: 83 to 99%) in subjects without bronchoscopy-detected lung cancer. The authors concluded that they developed a gene expression classifier measured in bronchial airway epithelial cells that is able to accurately identify lung cancer in current and former smokers who have undergone bronchoscopy for suspicion of lung cancer. They stated that due to the high NPV of the classifier, it could potentially inform clinical decisions regarding the need for further invasive testing in patients whose bronchoscopy is non-diagnostic.

Ferguson et al (2016) noted that bronchoscopy is frequently used for the evaluation of suspicious pulmonary lesions found on computed tomography, but its sensitivity for detecting lung cancer is limited. Recently, a bronchial genomic classifier was validated to improve the sensitivity of bronchoscopy for lung cancer detection, demonstrating a high sensitivity and negative predictive value (NPV) among patients at intermediate risk (10 to 60%) for lung cancer with an inconclusive bronchoscopy. These researchers examined if a negative genomic classifier result that down-classifies a patient from intermediate risk to low risk (less than 10%) for lung cancer would reduce the rate that physicians recommend more invasive testing among patients with an inconclusive bronchoscopy. These researchers conducted a randomized, prospective, decision impact survey study assessing pulmonologist recommendations in patients undergoing work-up for lung cancer who had an inconclusive bronchoscopy. Cases with an intermediate pretest risk for lung cancer were selected
from the AEGIS trials and presented in a randomized fashion to pulmonologists either with or without the patient's bronchial genomic classifier result to determine how the classifier results impacted physician decisions. A total of 202 physicians provided 1,523 case evaluations on 36 patients. Invasive procedure recommendations were reduced from 57% without the classifier result to 18% with a negative (low risk) classifier result ($p < 0.001$). Invasive procedure recommendations increased from 50 to 65% with a positive (intermediate risk) classifier result ($p < 0.001$). When stratifying by ultimate disease diagnosis, there was an overall reduction in invasive procedure recommendations in patients with benign disease when classifier results were reported (54 to 41%, $p < 0.001$). For patients ultimately diagnosed with malignant disease, there was an overall increase in invasive procedure recommendations when the classifier results were reported (50 to 64%, $p = 0.003$). The authors concluded that these findings suggested that a negative (low risk) bronchial genomic classifier result reduces invasive procedure recommendations following an inconclusive bronchoscopy and that the classifier overall reduced invasive procedure recommendations among patients ultimately diagnosed with benign disease. They stated that these results support the potential clinical utility of the classifier to improve management of patients undergoing bronchoscopy for suspect lung cancer by reducing additional invasive procedures in the setting of benign disease.

Vachani et al (2016) stated that bronchoscopy is often the initial diagnostic procedure performed in patients with pulmonary lesions suggestive of lung cancer. A bronchial genomic classifier was previously validated to identify patients at low risk for lung cancer after an inconclusive bronchoscopy. In this study, these investigators evaluated the potential of the classifier to reduce invasive procedure utilization in patients with suspected lung cancer. In 2 multi-center trials of patients undergoing bronchoscopy for suspected lung cancer, the classifier was measured in normal-appearing bronchial epithelial cells from a main-stem bronchus. Among patients with low and intermediate pretest probability of cancer ($n = 222$), subsequent invasive procedures after an inconclusive bronchoscopy were identified.
Estimates of the ability of the classifier to reduce unnecessary procedures were calculated. Of the 222 patients, 188 (85%) had an inconclusive bronchoscopy and follow-up procedure data available for analysis; 77 (41%) patients underwent an additional 99 invasive procedures, which included surgical lung biopsy in 40 (52%) patients. Benign and malignant diseases were ultimately diagnosed in 62 (81%) and 15 (19%) patients, respectively. Among those undergoing surgical biopsy, 20 (50%) were performed in patients with benign disease. If the classifier had been used to guide decision making, procedures could have been avoided in 50% (21 of 42) of patients undergoing further invasive testing. Furthermore, among 35 patients with an inconclusive index bronchoscopy who were diagnosed with lung cancer, the sensitivity of the classifier was 89%, with 4 (11%) patients having a false-negative classifier result. The authors concluded that invasive procedures after an inconclusive bronchoscopy occur frequently, and most are performed in patients ultimately diagnosed with benign disease. They stated that using the genomic classifier as an adjunct to bronchoscopy may reduce the frequency and associated morbidity of these invasive procedures.

UpToDate reviews on “Overview of the initial evaluation, diagnosis, and staging of patients with suspected lung cancer” (Thomas and Gould, 2017a), “Selection of modality for diagnosis and staging of patients with suspected non-small cell lung cancer” (Thomas and Gould, 2017b), and “Overview of the initial evaluation, treatment and prognosis of lung cancer” (Midthun, 2017) do not mention the use of genomic testing/classifier.

Also, an UpToDate review on “Procedures for tissue biopsy in patients with suspected non-small cell lung cancer” (Thomas and Gould, 2017c) states that “Although obtaining samples of lavage fluid or tissue for genomic analysis has been studied as a potential diagnostic tool designed to enhance the sensitivity of bronchoscopy for the diagnosis of lung cancer, further study is required before it can be recommended for routine use”.

mention the use of genomic testing/classifier.

**SelectMDx**

SelectMDx is a reverse transcription PCR (RT-PCR) assay performed on post-DRE, first-void urine specimens from patients with clinical risk factors for PCa, who are being considered for biopsy. The test measures the mRNA levels of the DLX1 and HOXC6 biomarkers, using KLK3 expression as internal reference, to aid in patient selection for prostate biopsy. Higher expression levels of DLX1 and HOXC6 mRNA are associated with an increased probability for high-grade (Gleason score (GS) greater than or equal to 7) prostate cancer. SelectMDx provides the likelihood of detecting PCa upon biopsy, and the probability for high-grade versus low-grade disease, with an AUC of 0.89 (95 % CI: 0.86 to 0.92).

Carlsson and Roobol (2017) provided an overview of the current state of the evidence and highlight recent advances in the evaluation and diagnosis of clinically significant PCa, focusing on biomarkers, risk calculators and multi-parametric MRI (mpMRI). In 2017 there are numerous options to improve early detection as compared to a purely PSA-based approach. All have strengths and drawbacks. In addition to repeating the PSA and performing clinical work-up (DRE and estimation of prostate volume), additional tests investigated in the initial biopsy setting are: % free PSA, PHI, 4Kscore, SelectMDx, and Michigan Prostate Score and in the repeat setting: % free PSA, PHI, 4Kscore, Prostate Cancer Antigen 3, and ConfirmMDx. Risk calculators are available for both biopsy settings and incorporate clinical data with, or without, biomarkers; mpMRI is an important diagnostic adjunct. The authors concluded that there are numerous tests available that can help increase the specificity of PSA, in the initial and repeat biopsy setting; they all coincide with a small decrease in sensitivity of detecting high-grade cancer. They noted that cost-effectiveness is crucial; and the way forward is a multi-variable risk assessment on the basis of readily available clinical data, potentially with the addition of PSA sub-forms, preferably at low cost; MRI in the pre-diagnostic setting is promising, but is not ready for “prime time”.
Hendriks and colleagues (2017) noted that the diagnosis of PCa is currently based on serum PSA testing and/or abnormal DRE and histopathologic evaluation of prostate biopsies. The main drawback of PSA testing is the lack of specificity for PCa. To improve early detection of PCa more specific biomarkers are needed. In the past few years, many new promising biomarkers have been identified; however, to-date, only a few have reached clinical practice. These researchers discussed new blood-based and urinary biomarker models that are promising for usage in PCa detection, follow-up and treatment decision-making. These include PHI, PCA3, 4-kallikrein panel (4K), transmembrane protease serine 2-ERG (TMPRSS2-ERG), ExoDx Prostate Intelliscore, SelectMDx and the Mi-Prostate score. Only few head-to-head studies are available for these new fluid-based biomarkers and/or models. The blood-based PHI and urinary PCA3 are 2 FDA-approved biomarkers for diagnosis of PCa. These investigators also provided an overview of published studies comparing these 2 available biomarkers for prediction of (i) PCa upon prostate biopsy, (ii) pathological features in radical prostatectomy specimen, and (iii) significant PCa in patients eligible for active surveillance. Studies showed opposing results in comparison of PHI with PCA3 for prediction of PCa upon initial and repeat prostate biopsy; PHI and PCA3 are able to predict pathological findings on radical prostatectomy specimen, such as tumor volume and Gleason score. Only PHI could predict seminal vesicle invasion and only PCA3 could predict multi-focality. There is some evidence that PHI outperformed PCA3 in predicting significant PCa in an active surveillance population. The authors concluded that future research should focus on independent validation of promising fluid-based biomarkers/models, and prospective comparison of biomarkers with each other.

Dijkstra and associates (2017) examined the cost-effectiveness of a new urinary biomarker-based risk score (SelectMDx; MDxHealth, Inc., Irvine, CA) to identify patients for TRUS-guided biopsy and to compare this with the current standard of care (SOC), using only PSA to select for TRUS-guided biopsy. A decision-tree and Markov model were developed to evaluate the cost-effectiveness of SelectMDx as a reflex test versus SOC in men with a PSA level of greater than 3 ng/ml. Transition probabilities,
utilities and costs were derived from the literature and expert opinion. Cost-effectiveness was expressed in QALYs and healthcare costs of both diagnostic strategies, simulating the course of patients over a time horizon representing 18 years. Deterministic sensitivity analyses were performed to address uncertainty in assumptions. A diagnostic strategy including SelectMDx with a cut-off chosen at a sensitivity of 95.7 % for high-grade PCa resulted in savings of €128 and a gain of 0.025 QALY per patient compared to the SOC strategy. The sensitivity analyses showed that the disutility assigned to active surveillance had a high impact on the QALYs gained and the disutility attributed to TRUS-guided biopsy only slightly influenced the outcome of the model. The authors concluded that based on the currently available evidence, the reduction of over-diagnosis and over-treatment due to the use of the SelectMDx test in men with PSA levels of greater than 3 ng/ml may lead to a reduction in total costs per patient and a gain in QALYs.

An UpToDate review on “Prostate biopsy” (Benway and Andriole, 2017) does not mention SelectMDx.

Furthermore, NCCN’s clinical practice guideline on “Prostate cancer” (Version 2.2017) does not mention SelectMDx as a management tool.

**ExoDx Prostate (IntelliScore):**

ExoDx Prostate (IntelliScore) is a non-invasive urine-based liquid biopsy for PCa. It is used to identify high-grade prostate cancer (HGPCA) both at the time of biopsy and at surgery. ExoDx Prostate is an exosomal RNA (exoRNA)-based assay that can be used prior to initial biopsy as well as for sequential monitoring of disease progression in patients enrolled in active surveillance.

Di Meo and associates (2017) noted that there is a growing trend towards exploring the use of a minimally invasive "liquid biopsy" to identify biomarkers in a number of cancers, including urologic malignancies. Multiple aspects can be assessed in circulating cell-free DNA, including cell-free DNA levels, integrity, methylation and mutations. Other prospective liquid biopsy markers include...
circulating tumor cells, circulating RNAs (microRNA [miRNA], long non-coding RNAs [lncRNAs] and messenger RNA [mRNA]), cell-free proteins, peptides and exosomes have also emerged as non-invasive cancer biomarkers. These circulating molecules can be detected in various biological fluids, including blood, urine, saliva and seminal plasma. Liquid biopsies hold great promise for personalized medicine due to their ability to provide multiple non-invasive global snapshots of the primary and metastatic tumors. The authors noted that although a promising source of cancer biomarkers, few exosomal biomarkers have been implemented into clinical practice. This is partly due to the lack of accurate isolation and detection methods. They speculate that the development of sensitive capture platforms is likely to trigger the introduction of novel exosomal biomarkers into the clinic in the near future.

Panigrahi and Deep (2017) noted that African American men in the US have higher incidence and mortality rates due to PCa compared to other races. In 2016 alone, nearly 30,000 cases of PCa in African American men were diagnosed and 4,450 men died from PCa. The underlying reasons for this health disparity in PCa are complex and include social, economic, and biologic determinants. To reduce or eliminate this health disparity, one must better understand the biology of the disease in African Americans and then develop novel diagnostic and prognostic biomarkers useful for timely and effective treatment decisions. Recently, there has been remarkable progress in understanding the role of exosomes (vesicles of 30 to 150 nm diameter) in cancer development and progression. Exosomes are loaded with unique cargo, including proteins, nucleic acids, lipids, and metabolites, that could predict the cells of their origin. Thus, circulating exosomes in cancer patients are being used as a type of biopsy to identify novel biomarkers for early diagnosis, prognosis, and therapeutics. The authors discussed the promising use of exosomes to (i) identify race-related unique biological features of PCa, and (ii) discover novel biomarkers for better diagnosis and prognosis of PCa, with the goal of reducing cancer health disparities.

Foj and colleagues (2017) stated that miRNAs are non-coding
small RNAs, involved in post-transcriptional regulation of many
target genes. In this study, 5 miRNAs that have been consistently
found deregulated in PCa (miR-21, miR-141, miR-214, miR-375,
and let-7c) were analyzed in urinary pellets from 60 PCa patients
and 10 healthy subjects by qRT-PCR. Besides, urinary exosomes
were isolated by differential centrifugation and analyzed for those
miRNAs. Significant up-regulation of miR-21, miR-141, and miR-
375 was found comparing PCa patients with healthy subjects in
urinary pellets, while miR-214 was found significantly down-
regulated. Regarding urinary exosomes, miR-21 and miR-375
were also significantly up-regulated in PCa but no differences
were found for miR-141. Significant differences were found for
let-7c in PCa in urinary exosomes while no differences were
observed in urinary pellets. A panel combining miR-21 and miR-
375 is suggested as the best combination to distinguish PCa
patients and healthy subjects, with an AUC of 0.872.

Furthermore, the association of miRNAs with clinicopathological
characteristics was investigated. MiR-141 resulted significantly
correlated with Gleason score in urinary pellets and let-7c with
clinical stage in urinary exosomes. Additionally, miR-21, miR-141,
and miR-214 were found significantly deregulated in
intermediate/high-risk PCa versus low-risk/healthy subjects in
urinary pellets. Significant differences between both groups were
found in urinary exosomes for miR-21, miR-375, and let-7c. The
authors concluded that these findings suggested that the analysis
of miRNAs-especially miRNA-21 and miR-375- in urine could be
useful as biomarkers in PCa.

Yang and co-workers (2017) stated that exosomes are membrane-
bound extracellular vesicles involved in intercellular
communication and tumor cell metastasis. In this study, flow
field-flow fractionation (FIFFF) was utilized to separate urinary
exosomes by size, demonstrating a significant difference in
exosome sizes between healthy controls and patients with PCa.
Exosome fractions of different sizes were collected for
microscopic analysis during an FIFFF run and evaluated with
exosome marker proteins using Western blot analysis. The results
indicated that exosomes of different sizes originated from
different types of cells. Collected exosome fractions were further
examined using nanoflow ultrahigh performance liquid
chromatography-electrospray ionization-tandem mass spectrometry (nUPLC-ESI-MS/MS) for lipidomic analysis. A total of 162 lipids (from 286 identified) were quantified using a selected reaction monitoring (SRM) method. The overall amount of lipids increased by 1.5- to 2-fold in patients with PCa and degree of increase was more significant in the smaller fractions (diameter less than 150 nm) than in the larger ones (diameter greater than 150 nm) some classes of lipids. In addition, neutral lipids like diacylglycerol (DAG) and triacylglycerol (TAG) decreased in all exosomes without size dependency. Moreover, a dramatic increase in 22:6/22:6-phosphatidyglycerol (PG) was observed and significant decrease in (16:0, 16:0)- and (16:1, 18:1)-DAG species (nearly 5-fold) and high abundant TAG species (greater than 2.5-fold) was observed in patients with PCa. The authors concluded that the findings of this study indicated that FIFFF can be employed for the high-speed screening of urinary exosome sizes in patients with PCa and lipidomic analysis of the fractionated exosomes has potential for developing and distinguishing biomarkers of PCa.

Pan and colleagues (2017) stated that exosomes are small vesicular bodies released by a variety of cells. Exosomes contain miRNAs, mRNAs and proteins with the potential to regulate signaling pathways in recipient cells. Exosomes deliver nucleic acids and proteins to mediate the communication between cancer cells and stroma cells. These investigators summarized recent progress in the understanding of the role of exosomes in PCa. The tumorigenesis, metastasis and drug resistance of PCa are associated with the cargos of exosomes such as miRNA, IncRNAs and proteins. In addition, PCa cells modulate surrounding stromal cells via the exosomes. Affected stromal cells employ the exosomes to modulate microenvironment and promote tumor growth and metastasis. Exosomes derived from PCa cells contribute to cancer chemo-resistance. The lipid bilayer membrane of the exosomes makes them promising carriers of drugs and other therapeutic molecules targeting PCa. Furthermore, exosomes can be detected and isolated from various body fluids for the diagnosis of PCa. The authors concluded that accumulating evidences confirm that exosomes are implicated in the progression and metastasis of PCa. Many
biological molecules are encapsulated in the exosomes from PCa such as miRNAs, IncRNAs and proteins, and their expression levels differ from those of normal prostate cells. The easy isolation of exosomes from body fluid enables them as potential biomarkers of PCa. Furthermore, the lipid bilayer membrane of exosomes makes them promising carriers of drugs and other therapeutic molecules to target PCa. In the near future, it is expected that the power of this nano-sized vesicles would be realized to promote the clinical application of exosomes in PCa diagnosis and therapy.

**Next-Generation Sequencing and Solid Cancers:**

Forouzanfar and associates (2017) noted that esophageal squamous cell carcinoma is one of the deadliest of all the cancers. Its metastatic properties portend poor prognosis and high rate of recurrence. A more advanced method to identify new molecular biomarkers predicting disease prognosis can be whole exome sequencing (WES). These researchers reported the most effective genetic variants of the Notch signaling pathway in esophageal SCC susceptibility by WES. These investigators analyzed 9 probands in unrelated familial esophageal SCC pedigrees to identify candidate genes. Genomic DNA was extracted and WES performed to generate information about genetic variants in the coding regions. Bioinformatics software applications were utilized to exploit statistical algorithms to demonstrate protein structure and variants conservation. Polymorphic regions were excluded by false-positive investigations. Gene-gene interactions were analyzed for Notch signaling pathway candidates. These researchers identified novel and damaging variants of the Notch signaling pathway through extensive pathway-oriented filtering and functional predictions, which led to the study of 27 candidate novel mutations in all 9 patients. Detection of the tri-nucleotide repeat containing 6B gene mutation (a slice site alteration) in 5 of the 9 probands, but not in any of the healthy samples, suggested that it may be a susceptibility factor for familial esophageal SCC. Noticeably, 8 of 27 novel candidate gene mutations (e.g., epidermal growth factor, signal transducer and activator of transcription 3, MET) act in a cascade leading to cell survival and proliferation. The authors
concluded that these findings suggested that the tri-nucleotide repeat containing 6B mutation may be a candidate predisposing gene in esophageal SCC. In addition, some of the Notch signaling pathway genetic mutations may act as key contributors to esophageal SCC.

Kyrochristos and colleagues (2017) stated that hepatobiliary and pancreatic (HBP) cancers are associated with high cancer-related death rates. Surgery aiming for complete tumor resection (R0) remains the cornerstone of the treatment for HBP cancers. The current progress in the adjuvant treatment is quite slow, with gemcitabine chemotherapy available only for pancreatic ductal adenocarcinoma (PDA). In the advanced and metastatic setting, only 2 targeted drugs have been approved by the FDA, which are sorafenib for hepatocellular carcinoma and erlotinib for PDA. It is a pity that multiple phase III randomized control trials (RCTs) examining the effectiveness of targeted agents have negative results. Failure in the development of effective drugs probably reflects the poor understanding of genome-wide alterations and molecular mechanisms orchestrating therapeutic resistance and recurrence. In the post-ENCODE (Encyclopedia of DNA Elements) era, cancer is referred to as a highly heterogeneous and systemic disease of the genome. The unprecedented potential of NGS technologies to accurately identify genetic and genomic variations has attracted major research and clinical interest. The applications of NGS include targeted NGS with potential clinical implications, while WES and whole-genome sequencing (WGS) focus on the discovery of both novel cancer driver genes and therapeutic targets. These advances dictate new designs for clinical trials to validate biomarkers and drugs. The authors discussed the findings of available NGS studies on HBP cancers and the limitations of genome sequencing analysis to translate genome-based biomarkers and drugs into patient care in the clinic. They concluded that the validity of NGS technologies to identify tumor heterogeneity-associated therapeutic resistance and relapse gives rise to high expectations for translating these advances into patient-centric trials and clinical benefit. In the medium-term, targeted NGS enables the conduction of umbrella and basket clinical trials. The identification of mutated or amplified gene-based patient subgroups and the subsequent
tumor-guided treatment with targeted drugs from the list of available FDA-approved agents, matching these specific genetic alterations, could improve personalized patient care. By contrast, the discovery of novel therapeutic targets by WES and WGS studies raises much higher expectations to substantially broaden the targeted drugs catalogue with a long-term perspective. However, this concept requires evaluation and confirmation by appropriately designed large-scale clinical trials. These researchers stated that targeted NGS, WES, and WGS could enable the development of robust biomarkers for tailored treatment; and translational NGS research represents a top prospect for faster progress than any other available technology to achieve precision oncology.

Lianos and co-workers (2017) noted that by identifying cancer driver genes involved in tumorigenesis, WES analyses enable the development of robust biomarkers and novel therapeutic targets to reach precision oncology. In this study, WES analyses were performed in matched gastric cancer-normal gastric tissues from 2 patients. These researchers compared genes highlighted with those of a database and recent WES/WGS studies. They identified 32 highlighted gastric cancer genes, 2 of these (DEFB118 and RNF43) may provide future potential clinical implications. The authors concluded that definitive evidence on extensive genetic heterogeneity suggested the need for large-scale NGS studies to validate gastric cancer driver genes catalog. This list represents the foundation for developing genome-based biomarkers to guide precision gastric cancer treatment.

Appendix

Glossary of Terms:

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>a2-PAG</td>
<td>Pregnancy-associated alpha2 glycoprotein</td>
</tr>
<tr>
<td>BCM</td>
<td>Breast cancer mucin</td>
</tr>
<tr>
<td>BTA</td>
<td>Bladder tumor antigen</td>
</tr>
<tr>
<td>CA19-9</td>
<td>Cancer antigen 19-9</td>
</tr>
<tr>
<td>CA50</td>
<td>Cancer antigen 50</td>
</tr>
<tr>
<td>Code</td>
<td>Description</td>
</tr>
<tr>
<td>------</td>
<td>-----------------------------------</td>
</tr>
<tr>
<td>CA72-4</td>
<td>Cancer antigen 72-4</td>
</tr>
<tr>
<td>CA195</td>
<td>Cancer antigen 195</td>
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<tr>
<td>CA242</td>
<td>Cancer antigen 242</td>
</tr>
<tr>
<td>CA549</td>
<td>Cancer antigen 549</td>
</tr>
<tr>
<td>SCC</td>
<td>Squamous cell carcinoma</td>
</tr>
<tr>
<td>CAM17-1</td>
<td>Monoclonal antimucin antibody 17-1</td>
</tr>
<tr>
<td>CAM26</td>
<td>Monoclonal antimucin antibody 26</td>
</tr>
<tr>
<td>CAM29</td>
<td>Monoclonal antimucin antibody 29</td>
</tr>
<tr>
<td>CAR3</td>
<td>Antigenic determinant recognized by monoclonal antibody AR3</td>
</tr>
<tr>
<td>DU-PAN-2</td>
<td>Sialylated carbohydrate antigen DU-PAN-2</td>
</tr>
<tr>
<td>FDP</td>
<td>Fibrin/fibrinogen degradation products</td>
</tr>
<tr>
<td>GCC</td>
<td>Guanylyl cyclase C</td>
</tr>
<tr>
<td>MCA</td>
<td>Mucin-like carcinoma-associated antigen</td>
</tr>
<tr>
<td>NMP22</td>
<td>Nuclear matrix protein22</td>
</tr>
<tr>
<td>NSE</td>
<td>Neuron-specific enolase PLAP</td>
</tr>
<tr>
<td>PNA-ELLA</td>
<td>Peanut lectin-bonding assay</td>
</tr>
<tr>
<td>SLEX</td>
<td>Sialylated Lewis X-antigen</td>
</tr>
<tr>
<td>SLX</td>
<td>Sialylated SSEA-1 antigen</td>
</tr>
<tr>
<td>SPAN-1</td>
<td>Sialylated carbonated antigen SPAN-1</td>
</tr>
<tr>
<td>ST-439</td>
<td>Sialylated carbonated antigen ST-439</td>
</tr>
<tr>
<td>TAG12</td>
<td>Tumor-associated glycoprotein 12</td>
</tr>
<tr>
<td>TAG72</td>
<td>Tumor-associated glycoprotein 72</td>
</tr>
<tr>
<td>TAG72.3</td>
<td>Tumor-associated glycoprotein 72.3</td>
</tr>
<tr>
<td>TATI</td>
<td>Tumor-associated trypsin inhibitor</td>
</tr>
<tr>
<td>TNF-a</td>
<td>Tumor necrosis factor alpha</td>
</tr>
<tr>
<td>TPA</td>
<td>Tissue polypeptide antigen</td>
</tr>
</tbody>
</table>

**CPT Codes / HCPCS Codes / ICD-10 Codes**
Information in the [brackets] below has been added for clarification purposes. Codes requiring a 7th character are represented by "+":

**Prostate-specific antigen (PSA):**

CPT codes covered if selection criteria are met:

- 84152 Prostate specific antigen (PSA);complexed (direct measurement)
- 84153 total
- 84154 free

CPT codes not covered for indications listed in the CPB:

- 81313 PCA3/KLK3 (prostate cancer antigen 3 [non-protein coding]/kallikrein-related peptidase 3 [prostate specific antigen]) ratio (eg, prostate cancer)

HCPCS codes covered if selection criteria are met:

- G0103 Prostate cancer screening; prostate specific antigen test (PSA)

ICD-10 codes covered if selection criteria are met:

- C61 Malignant neoplasm of prostate
- D07.5 Carcinoma in situ of prostate
- D40.0 Neoplasm of uncertain behavior of prostate
- R97.20 Elevated prostate specific antigen [PSA]
- R97.21
- Z12.5 Encounter for screening for malignant neoplasm of prostate
- Z85.46 Personal history of malignant neoplasm of prostate

ICD-10 codes not covered for indications listed in the CPB:

- C50.011 - C50.929 Malignant neoplasm of breast
- D05.00 - D05.92 Carcinoma in situ of breast
- D24.1 - D24.9 Benign neoplasm of breast
- D48.60 - D48.62 Neoplasm of uncertain behavior of breast
- D49.3 Neoplasm of unspecified behavior of breast
Z12.39    Encounter for other screening for malignant neoplasm of breast

*Carcinoembryonic antigen (CEA):*

**CPT codes covered if selection criteria are met:**

82378    Carcinoembryonic antigen (CEA)

**ICD-10 codes covered if selection criteria are met:**

C18.0 - C20  Malignant neoplasm of colon, rectosigmoid junction and rectum

C22.1      Intrahepatic bile duct carcinoma [cholangiocarcinoma]

C23 - C24.9 Malignant neoplasm of gallbladder and other and unspecified parts of biliary tract

C25.0 -     Malignant neoplasm of pancreas

C25.9

C34.00 -    Malignant neoplasm of bronchus and lung

C34.92

C50.011 -   Malignant neoplasm of breast

C50.929

C56.1 -     

C56.9

C73        Malignant neoplasm of thyroid gland [medullary thyroid cancer]

C80.0 -     Disseminated and other malignant neoplasm, unspecified

C80.1

D01.0      Carcinoma in situ of colon

D01.5      Carcinoma in situ of liver, gallbladder and bile ducts

D02.20 -   Carcinoma in situ of bronchus and lung

D02.22

D05.00 -   Carcinoma in situ of breast

D05.92

D07.39     Carcinoma in situ of other female genital organs [ovary]

D09.3      Carcinoma in situ of thyroid and other endocrine glands

D13.4      Benign neoplasm of liver [intrahepatic bile ducts]
D13.6  Benign neoplasm of pancreas
D13.7  Benign neoplasm of endocrine pancreas [Benign neoplasm of islets of Langerhans]
D24.1  Benign neoplasm of breast
D24.9
D27.0  Benign neoplasm of ovary
D27.9
D34  Benign neoplasm of thyroid gland
K86.2  Cyst and pseudocyst of pancreas
K86.3
R17  Carcinoma in situ of other female genital organs [ovary]
R93.2  Abnormal findings on diagnostic imaging of liver and biliary tract
R94.5  Abnormal results of liver function studies
Z85.030  Personal history of malignant neoplasm of large intestine, rectum, rectosigmoid junction, and anus
Z85.048

**ICD-10 codes not covered for indications listed in the CPB:**

C15.3  Malignant neoplasm of esophagus
C15.9
D48.60  Neoplasm of uncertain behavior of breast
D48.62
D49.3  Neoplasm of unspecified behavior of breast
Z12.2  Encounter for screening for malignant neoplasm of respiratory organs
Z12.11  Encounter for screening for malignant neoplasm of colon and rectum
Z12.12
Z12.39  Encounter for other screening for malignant neoplasm of breast

**Adenomatous polyposis coli (APC):**

**CPT codes covered if selection criteria are met:**

81201  APC (adenomatous polyposis coli) (eg, familial adenomatosis polyposis [FAP], attenuated FAP) gene analysis
81203
ICD-10 covered if selection criteria are met:

D12.0  -  Benign neoplasm of colon
D12.9

D48.1  -  Neoplasm of uncertain behavior of connective and other soft tissue [desmoid fibromatosis]

Z83.71  -  Family history of colonic polyps

*Afirma Thyroid FNA analysis - No specific code:*

ICD-10 codes covered if selection criteria are met:

D44.0  -  Neoplasm of uncertain behavior of thyroid gland [indeterminate thyroid nodules] [not covered for repeat testing of indeterminate thyroid nodules]

E04. 0  -  Other nontoxic goiter
E04.9

*FISH assay of the BCR/ABL gene:*

CPT codes covered if selection criteria are met:

81206  -  BCR/ABL1 (t(9;22)) (eg, chronic myelogenous leukemia) translocation analysis
81208  -  Molecular cytogenetics; DNA probe, each (eg, FISH)
88271  -  interphase in situ hybridization, analyze 100-300 cells
88275  -  Lymphoblastic (diffuse) lymphoma

ICD-10 codes covered if selection criteria are met:

C83.50  -  Chronic lymphocytic leukemia of B-cell type
C91.10  -  Myeloid leukemia
C91.12
C92.00  -  Cancer antigen 125 (CA 125):
C92.12

CPT codes covered if selection criteria are met:

86304  -  Immunoassay for tumor antigen, quantitative; CA 125

ICD-10 codes covered if selection criteria are met:

C56.1  -  Malignant neoplasm of ovary
C56.9
D39.10 - Neoplasm of uncertain behavior of ovary
D39.12
Z12.73 - Encounter for screening for malignant neoplasm of ovary
Z80.41 - Family history of malignant neoplasm of ovary

ICD-10 codes not covered for indications listed in the CPB:
Z12.11 - Encounter for screening for malignant neoplasm of colon and rectum
Z12.12 -
Z85.43 - Personal history of malignant neoplasm of ovary

Serial measurements of CA 15-3 (also known as CA 27-29 or Truquant RIA):

CPT codes covered if selection criteria are met:
86300 - Immunoassay for tumor antigen, quantitative; CA 15-3 (27.29)

ICD-10 codes covered if selection criteria are met:
C50.011 - Malignant neoplasm of the female breast
C50.019
C50.111 -
C50.119
C50.211 -
C50.219
C50.311 -
C50.319
C50.411 -
C50.419
C50.511 -
C50.519
C50.611 -
C50.619
C50.811 -
C50.819
C50.911 -
C50.919
D05.00 - Carcinoma in situ of breast
D05.92
Z85.3 - Personal history of malignant neoplasm of breast
ICD-10 codes not covered for indications listed in the CPB:

Z12.31 - Encounter for screening for malignant neoplasm of breast
Z12.39

CA 19-9:

CPT codes covered if selection criteria are met:

86301 Immunoassay for tumor antigen, quantitative; CA 19-9

ICD-10 codes covered if selection criteria are met:

C16.0 - Malignant neoplasm of stomach
C16.9

C18.1 Malignant neoplasm of appendix [mucinous appendiceal carcinoma]
C22.1 Intrahepatic bile duct carcinoma [cholangiocarcinoma]
C23 - C24.9 Malignant neoplasm of gallbladder and other and unspecified parts of biliary tract
C25.0 - Malignant neoplasm of pancreas
C25.9

D00.2 Carcinoma in situ of stomach
D01.5 Carcinoma in situ of liver, gallbladder and bile ducts [covered for gallbladder and bile duct]
D01.7 - Carcinoma in situ of other and unspecified digestive organs
D01.9

R17 Unspecified jaundice
R93.2 Abnormal findings on diagnostic imaging of liver and biliary tract
R94.5 Abnormal results of liver function studies
Z76.82 Awaiting organ transplant status
Z85.028 Personal history of other malignant neoplasm of stomach
Z85.07 - Personal history of malignant neoplasm of pancreas
Z85.09 and other digestive organs

ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):

C15.3 - Malignant neoplasm of esophagus
C15.9
C18.0 - C20  Malignant neoplasm of colon, rectosigmoid junction and rectum

C22.0, C22.2  Malignant neoplasm of liver
- C22.9

C50.011 - C50.929  Malignant neoplasm of the breast

C53.0 - C55, C58  Malignant neoplasm of uterus

D01.0  Carcinoma in situ of colon

D01.5  Carcinoma in situ of liver, gallbladder and bile ducts
[not covered for liver]

D05.00 - D05.92  Carcinoma in situ of breast

K86.2 - K86.3  Cyst and pseudocyst of pancreas

_{Cardioembryonic antigen cellular adhesion molecule-7 (CEACAM-7)}
- No specific code:

No specific code

_{ICD-10 codes not covered if selection criteria are met:}:

C19 - C21.8  Malignant neoplasm of rectum, rectosigmoid junction and anus

D01.1 - D01.2  Carcinoma in situ of rectosigmoid junction and rectum

Z85.048  Personal history of other malignant neoplasm of rectum, rectosigmoid junction, and anus

_{Molecular Intelligence Services, including MI Profile and MI Profile PLUS (formerly Target Now Molecular Profiling Test, including Target Now Select and Target Now Comprehensive)} - No specific code:

_{Cyfra21-1 (a cytokeratin 19 fragment), p53, & Squamous cell carcinoma antigen (SCC-Ag)} - No specific code:

_{ICD-10 codes not covered for indications listed in the CPB:}:

C15.3 - Malignant neoplasm of esophagus

C15.9
Vascular endothelial growth factor C (VEGF-C) - No specific code:

ICD-10 codes not covered for indications listed in the CPB:

C15.3 - Malignant neoplasm of esophagus
C15.9

Human epidermal growth factor receptor 2 (HER2) evaluation:

CPT codes covered if selection criteria are met:

83950 Oncoprotein; Her-2/neu

ICD-10 codes covered if selection criteria are met:

C15.3 - Malignant neoplasm of esophagus
C15.9
C16.0 - Malignant neoplasm of stomach
C16.9
C50.011 - Malignant neoplasm of breast [see criteria]
C50.929

ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):

C18.1 Malignant neoplasm of appendix

IGH@ (Immunoglobulin heavy chain locus):

CPT codes covered if selection criteria are met:

81261 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)

ICD-10 codes covered if selection criteria are met:

C85.10 - Other specified and unspecified types of non-Hodgkin lymphoma
C85.99
C91.40 - Hairy cell leukemia
C91.42
D47.z1 Post-transplant lymphoproliferative disorder (PTLD)
E85.9 Amyloidosis, unspecified [systemic light chain]

IGK@ (Immunoglobulin kappa light chain locus):

CPT codes covered if selection criteria are met:
IGK (Immunoglobulin kappa light chain locus) (eg, leukemia and lymphoma, B-cell), gene rearrangement analysis, evaluation to detect abnormal clonal population(s)

**ICD-10 codes covered if selection criteria are met:**

- C85.10 - Other specified and unspecified types of non-Hodgkin lymphoma
- C85.99 - Hairy cell leukemia
- C91.40 - C91.42 - Other specified and unspecified types of non-Hodgkin lymphoma
- E85.9 - Amyloidosis, unspecified [systemic light chain]

**Serial measurements of human chorionic gonadotropin (HCG):**

**CPT codes covered if selection criteria are met:**

- 84702 - Gonadotropin, chorionic (hCG); quantitative

**ICD-10 codes covered if selection criteria are met:**

- C56.1 - C56.9 - Malignant neoplasm of ovary
- C56.9 - Malignant neoplasm of placenta (e.g., choriocarcinoma)
- C62.00 - C62.92 - Malignant neoplasm of testis
- C62.92 - Secondary malignant neoplasm of intrathoracic lymph nodes [mediastinal nodes]
- D07.30 - Carcinoma in situ of other and unspecified female genital organs [germinal cell tumors (teratocarcinoma and embryonal cell carcinoma) of the ovaries] tumors (teratocarcinoma and embryonal cell carcinoma) of the ovaries]
- D07.60 - Carcinoma in situ of other and unspecified male genital organs
- D07.69 - Neoplasm of uncertain behavior of placenta
- D39.2 - Hydatidiform mole, unspecified
- Z85.43 - Personal history of malignant neoplasm of ovary
- Z85.47 - Personal history of malignant neoplasm of testis

**Serial measurements of AFP to diagnose germ cell tumors or the diagnosis and monitoring of hepatocellular carcinoma:**
CPT codes covered if selection criteria are met:

82105  Alpha-fetoprotein (AFP); serum

10 codes covered if selection criteria are met:

B17.10 - Acute hepatitis C without or with hepatic coma
B17.11
B18.2  Chronic viral hepatitis C
B19.20 - Unspecified viral hepatitis C without or with hepatic coma
B19.21
C22.0 - Malignant neoplasm of the liver and intrahepatic bile ducts
C22.9
C37  Malignant neoplasm of thymus
C56.1 - Malignant neoplasm of ovary
C56.9
C62.00 - Malignant neoplasm of testes
C62.92
C77.1  Secondary malignant neoplasm of intrathoracic lymph nodes [mediastinal nodes]
D01.5  Carcinoma in situ of liver, gallbladder and bile ducts
D07.30 - Carcinoma in situ of other and unspecified female genital organs [germ cell tumors]
D07.39
D07.60 - Carcinoma in situ of other and unspecified male genital organs
D07.69
D15.0  Benign neoplasm of thymus
E83.110  Hereditary hemochromatosis
E88.01  Alpha-1-antitrypsin deficiency
F10.10 - Alcohol related disorders
F10.99
K70.30 - Alcoholic cirrhosis of liver without or with ascites
K70.31
K74.3  Primary biliary cirrhosis brackets [stage 4 primary biliary cirrhosis]
K74.60 - Unspecified or other cirrhosis of liver
K74.69
K75.81  Nonalcoholic steatohepatitis (NASH)
Other specified disorders of male genital organs [testicular mass]

Intra-abdominal and pelvic swelling, mass, lump, unspecified site

Generalized and other intra-abdominal and pelvic swelling, mass and lump

Localized swelling, mass and lump, trunk

Encounter for screening for malignant neoplasm of other sites

Carrier of viral Hepatitis B

Family history of malignant neoplasm of digestive organs [family history of hepatocellular carcinoma]

Personal history of malignant neoplasm of ovary

Personal history of malignant neoplasm of testis

ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):

Malignant neoplasm of placenta (e.g., choriocarcinoma)

Neoplasm of uncertain behavior of placenta

Hydatidiform mole, unspecified

Serial measurements of AFP and HCG together to diagnose and monitor testicular cancer:

CPT codes covered if selection criteria are met:

Alpha-fetoprotein (AFP); serum

Gonadotropin, chorionic (hCG); quantitative

ICD-10 codes covered if selection criteria are met:

Malignant neoplasm of testes

Carcinoma in situ of other and unspecified male genital organs

Encounter for screening for malignant neoplasm of testis

Measurement of estrogen and progesterone receptors and steroid receptor status:
CPT codes covered if selection criteria are met:

84233  Receptor assay; estrogen
84234  progesterone

ICD-10 codes covered if selection criteria are met:

C50.011 - Malignant neoplasm of breast
C50.929
D05.00 - Carcinoma in situ of breast
D05.92

Targeted hematologic genomic sequencing panel (5-50 genes) for myelodysplastic syndromes:

CPT codes covered if selection criteria are met:

81450  Targeted genomic sequence analysis panel, hematolymphoid neoplasm or disorder, DNA analysis, and RNA analysis when performed, 5-50 genes (eg, BRAF, CEBPA, DNMT3A, EZH2, FLT3, IDH1, IDH2, JAK2, KRAS, KIT, MLL, NRAS, NPM1, NOTCH1), interrogation for sequence variants, and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed

ICD-10 codes covered if selection criteria are met:

D46.0 - Myelodysplastic syndromes
D46.9

Targeted hematologic genomic sequencing panel (5-50 genes) for non-small cell lung cancer:

CPT codes covered if selection criteria are met:

81445  Targeted genomic sequence analysis panel, solid organ neoplasm, DNA analysis, and RNA analysis when performed, 5-50 genes (eg, ALK, BRAF, CDKN2A, EGFR, ERBB2, KIT, KRAS, NRAS, MET, PDGFRA, PDGFRB, PGR, PIK3CA, PTEN, RET), interrogation for sequence variants and copy number variants or rearrangements, if performed

ICD-10 codes covered if selection criteria are met:

C34.00 - Malignant neoplasm of bronchus and lung [non-small cell]
**T-cell receptor gene rearrangements:**

**CPT codes covered if selection criteria are met:**

- **81340** TRB@ (T cell antigen receptor, beta) (eg, leukemia and lymphoma), gene rearrangement analysis to detect abnormal clonal population(s); using amplification methodology (eg, polymerase chain reaction)
- **81341** using direct probe methodology (eg, Southern blot)
- **81342** TRG@ (T cell antigen receptor, gamma) (eg, leukemia and lymphoma), gene rearrangement analysis, evaluation to detect abnormal clonal population(s)

**ICD-10 codes covered if selection criteria are met:**

- **C84.00 - C84.09** Mycosis fungoides
- **C84.10 - C84.19** Sezary disease
- **C84.40 - C84.49** Peripheral T-cell lymphoma, not classified
- **C84.49**
- **C86.0** Extramedial NK/T-cell lymphoma, nasal type
- **C86.6** Primary cutaneous CD30-positive T-cell lymphoproliferations
- **C91.60 - C91.62** Prolymphocytic leukemia of T-cell type
- **C91.62**
- **C91.20 - C91.22** Other lymphoid leukemia with bracketed info [T-cell large granular lymphocytic]
- **D47.22** Castleman's disease

*ThyGenX (formerly MirInform Thyroid), Thyroseq, ThyraMIR - No specific code:*

**ICD-10 codes covered if selection criteria are met:**

- **E04.0 - E04.9** Other nontoxic goiter [thyroid nodules] [not covered for repeat testing of indeterminate thyroid nodules]

*K-ras (KRAS) with BRAF reflex testing:*

**CPT codes covered if selection criteria are met:**

- **81210** BRAF (v-raf murine sarcoma viral oncogene homolog B1) (eg, colon cancer), gene analysis, V600E variant
KRAS (Kirsten rat sarcoma viral oncogene homolog) (eg, carcinoma) gene analysis; variants in exon 2 (eg, codons 12 and 13)

KRAS (Kirsten rat sarcoma viral oncogene homolog) (eg, carcinoma) gene analysis; additional variant(s) (eg, codon 61, codon 146)

Other CPT codes related to the CPB:

- 83891 Molecular diagnostics; isolation or extraction of highly purified nucleic acid, each nucleic acid type (ie, DNA or RNA)
- 83896 nucleic acid probe, each
- 83898 amplification, target, each nucleic acid sequence
- 83907 lysis of cells prior to nucleic acid extraction (eg, stool specimens, paraffin embedded tissue), each specimen
- 83909 separation and identification by high resolution technique (e.g., capillary electrophoresis), each nucleic acid preparation
- 83912 interpretation and report
- 88363 Examination and selection of retrieved archival (ie, previously diagnosed) tissue(s) for molecular analysis (eg, KRAS mutational analysis)

Other HCPCS codes related to the CPB:

- J9055 Injection, cetuximab, 10 mg [to predict non-response to cetuximab (Erbitux) and panitumumab (Vectibix) in the treatment of anal adenocarcinoma]
- J9303 Injection, panitumumab, 10 mg [to predict non-response to cetuximab (Erbitux) and panitumumab (Vectibix) in the treatment of anal adenocarcinoma]

ICD-10 codes covered if selection criteria are met:

- C17.0 - Malignant neoplasm of small intestine [small bowel adenocarcinoma]
- C17.9
- C18.0 - C20 Malignant neoplasm of colon, rectosigmoid junction and rectum [metastatic colorectal cancer]
- C21.0 - Malignant neoplasm of anal canal and anus [anal adenocarcinoma]
- C21.1
Malignant neoplasm of bronchus and lung

Carcinoma in situ of rectum [if KRAS nonmutated]

Benign neoplasm of rectum and anal canal [if KRAS nonmutated] [Lynch syndrome (HNPCC)]

Carcinoma in situ of rectum [under age 50]

MUC1 - no specific code:

ICD-10 codes not covered for indications listed in the CPB:

ALK Gene Fusion:

No specific code

ICD-10 codes covered if selection criteria are met:
C34.00 - Malignant neoplasm of bronchus and lung [non-small-cell cancer]
C34.92

**ALK Gene Rearrangement - No specific code:**

**ICD-10 codes covered if selection criteria are met:**

C83.30 - Diffuse large B-cell lymphoma
C83.39
C84.40 - Peripheral T-cell lymphoma, not classified
C84.49
D47.21 - Post-transplant lymphoproliferative disorder (PTLD)

**ALK Translocations - no specific code:**

**Other CPT codes related to CPB:**

81401 - 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)

**ICD-10 codes covered if selection criteria are met:**

C34.00 - Malignant neoplasm of bronchus and lung [non-small-cell lung cancer]
C34.92

**Urokinase plasminogen activator (uPA) and plasminogen activator inhibitor 1 (PAI-1):**

**CPT codes covered if selection criteria are met:**

85415 - Fibrinolytic factors and inhibitors; plasminogen activator

**ICD-10 codes covered if selection criteria are met:**

C50.011 - Malignant neoplasm of breast [node negative]
C50.929
D05.00 - Carcinoma in situ of breast
D05.92

**Veristrat - No specific code:**

**CPT codes not covered for indications listed in the CPB:**
Oncology (lung), mass spectrometric 8-protein signature, including amyloid A, utilizing serum, prognostic and predictive algorithm reported as good versus poor overall survival

**ICD-10 codes covered if selection criteria are met:**

- **C34.00 - C34.92**: Malignant neoplasm of bronchus and lung [for persons with advanced NSCLC, whose tumors are without EGFR and ALK mutations, who have progressed after at least one chemotherapy regimen, and for whom erlotinib is considered an appropriate treatment]

**CD 117 (c-kit):**

**CPT codes covered if selection criteria are met:**

- **81272**: 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)
- **81273**: KIT (v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog) (eg, mastocytosis), gene analysis, D816 variant(s)
- **88184**: Flow cytometry, cell surface, cytoplasmic, or nuclear marker, technical component only; first marker
  + **88185**: each additional marker (List separately in addition to code for first marker)

**ICD-10 codes covered if selection criteria are met:**

[for determining eligibility for treatment with Gleevac]

- **C15.3 - C15.9**: Malignant neoplasm of esophagus
- **C92.10 - C92.12**: Chronic myeloid leukemia, BCR/ABL-positive

**CD 20:**

**CPT codes covered if selection criteria are met:**

- **88184**: Flow cytometry, cell surface, cytoplasmic, or nuclear marker, technical component only; first marker
+ 88185 each additional marker (List separately in addition to code for first marker)

**ICD-10 codes covered if selection criteria are met:**

[for determining eligibility for treatment with Rituxan]

C81.00 - Malignant neoplasms of lymphoid, hematopoietic and related tissue
C86.6
C88.4
C91.10 -
C91.12
C91.40 -
C91.42
C96.0 -
C96.4
C96.a -
C96.9

**CD 25:**

**CPT codes covered if selection criteria are met:**

88184 Flow cytometry, cell surface, cytoplasmic, or nuclear marker, technical component only; first marker

+ 88185 each additional marker (List separately in addition to code for first marker)

**ICD-10 codes covered if selection criteria are met:**

[for determining eligibility for treatment with Ontak]

C84.00 - Mycosis fungoides, Sezary disease and peripheral T-cell lymphoma, not classified
C84.49

**CD 31 - No specific code:**

**Other CPT codes related to the CPB:**

88341 - Immunohistochemistry or immunocytochemistry, per specimen
88344

**ICD-10 codes covered if selection criteria are met:**

C49.0 - Malignant neoplasm of other connective and soft tissue [angiosarcoma]
C49.9

**CD 33:**

**CPT codes covered if selection criteria are met:**
88184 Flow cytometry, cell surface, cytoplasmic, or nuclear marker, technical component only; first marker
+ 88185 each additional marker (List separately in addition to code for first marker)

ICD-10 codes covered if selection criteria are met: [for determining eligibility for treatment with Mylotarg]

C91.00 - Acute lymphoblastic leukemia [ALL]
C91.02
C92.00 - Acute myeloid leukemia
C92.02
C92.40 -
C92.a2
C93.00 - Acute monoblastic/monocytic leukemia
C93.02
C94.00 - Acute erythroid leukemia
C94.02
C95.00 - Acute leukemia of unspecified cell type
C95.02

CD 52:

CPT codes covered if selection criteria are met:

88184 Flow cytometry, cell surface, cytoplasmic, or nuclear marker, technical component only; first marker
+ 88185 each additional marker (List separately in addition to code for first marker)

ICD-10 codes covered if selection criteria are met: [for determining eligibility for treatment with Campath]
C82.00 - C82.99  
C83.10 - C83.89  
C84.00 - C84.49  
C84.a0 - C84.99  
C85.10 - C86.6  
C91.10 - C91.12  
C91.40 - C91.42  

**Cyclin D1:**

**CPT codes covered if selection criteria are met:**

81401  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) (EML4/ALK inv(2)) (eg, non-small-cell lung cancer), translocation or inversion analysis

**ICD-10 codes covered if selection criteria are met:**

C83.10 - Mantle cell lymphoma [diagnosing and predicting disease recurrence]

**ICD-10 codes not covered for indications listed in the CPB:**

C44.02, Squamous cell carcinoma of lip, eyelid, ear and external canal, face, scalp and neck

**DecisionDx-UM - No specific code:**

**ICD-10 codes covered if selection criteria are met:**
C69.30 - Malignant neoplasm of choroid and ciliary body
C69.42 [localized uveal melanoma]

**Endopredict (12-gene score):**

**HCPCS codes covered if selection criteria are met:**
S3854 Gene expression profiling panel for use in the management of breast cancer treatment [EndoPredict]

**ICD-10 codes covered if selection criteria are met:**
C50.011 - Malignant neoplasm of breast
C50.929

**Fas-Associated Protein with Death Domain FADD - No specific code:**

**ICD-10 codes not covered for indications listed in the CPB:**
C44.02, Squamous cell carcinoma of lip, eyelid, ear and external canal, face, scalp and neck
C44.121,
C44.129,
C44.221 -
C44.229,
C44.320 -
C44.329,
C44.42

**Prostate PX, Post-op PX:**

**Other CPT codes related to the CPB:**
88305 Level IV - Surgical pathology, gross and micropscopic examination
88313 Special stain including interpretation and report; Group II, al other (eg, iron trichrome), except stain for microorganisms, stains for enzyme constituents, or immunocytochemistry and immunohistochemistry
88323 Consultation and report on referred material requiring preparation of slides
88341 - Immunohistochemistry or immunocytochemistry, per specimen
88344
88350 Immunofluorescence, per specimen; each additional single antibody stain procedure (List separately in addition to code for primary procedure)

**ICD-10 codes not covered for indications listed in the CPB:**
C61 Malignant neoplasm of prostate

**NRAS mutation:**

**CPT codes covered if selection criteria are met:**

81311 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)

**Other CPT codes related to the CPB:**

81404 Molecular pathology procedure, Level 5 (eg, analysis of 2-5 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 6-10 exons, or characterization of a dynamic mutation disorder/triplet repeat by Southern blot analysis)

**Ras oncogenes (except KRAS and BRAF) - No specific code:**

**Epidermal growth factor receptor (EGFR) Testing:**

**CPT codes covered if selection criteria are met:**

81235 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)

**Other CPT codes related to the CPB:**

83890 - 83912 88341 - 88344 88381 Molecular diagnostics Immunohistochemistry or immunocytochemistry, per specimen Microdissection (ie, sample preparation of microscopically identified target); manual

**ICD-10 codes covered if selection criteria are met:**

C34.00 - Malignant neoplasm of bronchus and lung [non small cell lung cancer]
C34.92

**ICD-10 codes not covered if selection criteria are met:**

C71.0 - Malignant neoplasm of brain [Glioblastoma multiforme]
C71.9
D09.0 Carcinoma in situ of bladder [urothelial carcinoma]
D09.10 - Carcinoma in situ of other and unspecified urinary organs (ureter, renal pelvis) [urothelial carcinoma]

ROS-1 - No specific code:

ICD-10 codes covered if selection criteria are met:
C34.00 - Malignant neoplasm of bronchus and lung [non small cell lung cancer]
C34.92

ZAP-70:

CPT codes covered if selection criteria are met:
88184  Flow cytometry, cell surface, cytoplasmic, or nuclear marker, technical component only; first marker
+ 88185  each additional marker (List separately in addition to code for first marker)

ICD-10 codes covered if selection criteria are met:
C91.10 - Chronic lymphocytic leukemia of B-cell type [assessing prognosis and need for aggressive therapy]
C91.12

Oncotype Dx:

CPT codes covered if selection criteria are met:
81519  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

CPT codes not covered for indications listed in the CPB:
81525  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

Other CPT codes related to the CPB:
88360  Morphometric analysis, tumor immunohistochemistry (eg, Her-2/neu, estrogen receptor/progesterone receptor), quantitative or semiquantitative, per specimen, each single antibody stain procedure; manual
88361  using computer-assisted technology
Morphometric analysis, in situ hybridization,
(quantitative or semi-quantitative)

**ICD-10 codes covered if selection criteria are met:**

- C50.011 - Malignant neoplasm of female breast [except node positive] [HER2-negative, estrogen-receptor positive, node-negative breast cancer]
- C50.019
- C50.111 -
- C50.119
- C50.211 -
- C50.219
- C50.311 -
- C50.319
- C50.411 -
- C50.419
- C50.511 -
- C50.519
- C50.611 -
- C50.619
- C50.811 -
- C50.819
- C50.911 -
- C50.919

C50.021 - Malignant neoplasm of male breast
- C50.029
- C50.121 -
- C50.129
- C50.221 -
- C50.229
- C50.321 -
- C50.329
- C50.421 -
- C50.429
- C50.521 -
- C50.529
- C50.621 -
- C50.629
- C50.821 -
- C50.829
- C50.921 -
- C50.929
C77.3 Secondary and unspecified malignant neoplasm of axilla and upper limb lymph nodes [1-3 involved ipsilateral axillary lymph nodes]

ICD-10 codes not covered for indications listed in the CPB:

- C18.0 - C20 Malignant neoplasm of colon, rectosigmoid junction and rectum
- C61 Malignant neoplasm of prostate
- D01.0 Carcinoma in situ of colon
- D05.10 - D05.12 Intraductal carcinoma in situ of breast
- D05.12
- D07.5 Carcinoma in situ of prostate
- Z85.030 - Z85.048 Personal history of malignant neoplasm of large intestine, rectum, rectosigmoid junction, and anus

*Myeloperoxidase (MPO) immunostaining FLT3-ITD, CEBPA mutation, NPM1 mutation and KIT mutation:*

CPT codes covered if selection criteria are met:

- 81245 - 81246 FLT3 (fms-related tyrosine kinase 3) (eg, acute myeloid leukemia), gene analysis
- 83876 Myeloperoxidase (MPO)

Other CPT codes related to the CPB:

- 83891 Molecular diagnostics; isolation or extraction of highly purified nucleic acid, each nucleic acid type (ie, DNA or RNA)
- 83892 enzymatic digestion, each enzyme treatment
- 83898 amplification target, each nucleic acid sequence
- 83900 amplification target, multiplex, first two nucleic acid sequences
- 83901 amplification target, multiplex, each additional nucleic acid sequence beyond 2 (list separately in addition to code for primary procedure)
- 83903 mutation scanning, by physical properties (eg, single strand conformational polymorphisms [SSCP], heteroduplex, denaturing gradient gel electrophoresis [DGGE], RNA'ase A), single segment, each
mutation identification by sequencing, single segment, each segment

separation and identification by high resolution technique (eg, capillary electrophoresis), each nucleic acid preparation

interpretation and report

ICD-10 codes covered if selection criteria are met:
C92.00 - Acute myeloid leukemia
C92.02
C92.40 -
C92.a2

**PDGFRG:**

CPT codes covered for indications listed in the CPB:
81314 PDGFRA (platelet-derived growth factor receptor, alpha polypeptide) (eg, gastrointestinal stromal tumor [GIST]), gene analysis, targeted sequence analysis (eg, exons 12, 18)

ICD-10 codes covered if selection criteria are met:
C49.4 Malignant neoplasm of connective and soft tissue of abdomen

**PML/RARA:**

CPT codes covered if selection criteria are met:
81315 - PML/RARalpha, (t(15;17)), (promyelocytic leukemia/retinoic acid receptor alpha) (eg, promyelocytic leukemia) translocation analysis
81316

ICD-10 codes covered if selection criteria are met:
C92.00 - Acute myeloblastic leukemia
C92.02

**Placental alkaline phosphatase (PLAP):**

CPT codes covered if selection criteria are met:
84080 Phosphatase, alkaline; isoenzymes

ICD-10 codes covered if selection criteria are met:
C56.1 - Malignant neoplasm of ovary
C56.9
C62.00 - Malignant neoplasm of testes
C62.92
D07.30 - Carcinoma in situ of other and unspecified female genital organs [germ cell tumors]
D07.39
D07.60 - Carcinoma in situ of other and unspecified male genital organs
D07.69
Z85.43 Personal history of malignant neoplasm of ovary
Z85.47 Personal history of malignant neoplasm of testis

Bladder tumor antigen (BTA) Stat Test, the nuclear matrix protein (NMP22) test, the fibrin/fibrinogen degradation products (Aura-Tek FDP) test, or the UroVysion fluorescent in situ hybridization (FISH) test, BTA TRAK:

CPT codes covered if selection criteria are met:

85362 - Fibrin degradation products
85380
86294 Immunoassay for tumor antigen, qualitative or semiquantitative (e.g., bladder tumor antigen)
86386 Nuclear Matrix Protein 22 (NMP22) qualitative
88120 Cytopathology, in situ hybridization (eg, FISH), urinary tract specimen with morphometric analysis, 3-5 molecular probes, each specimen; manual
88121 using computer-assisted technology
88364 - 88366 In situ hybridization (eg, FISH), each probe
88366

ICD-10 codes covered if selection criteria are met:

C67.0 - Malignant neoplasm of bladder
C67.9
D09.0 Carcinoma in situ of bladder
Z85.51 Personal history of malignant neoplasm of bladder

ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):

R31.0 - Hematuria
R31.9
Z12.6 Encounter for screening for malignant neoplasm of bladder
**ImmunoCyte (uCyt) - No specific code:**

ICD-10 codes covered if selection criteria are met:

C67.0 - Malignant neoplasm of bladder
C67.9

ICD-10 codes not covered for indications listed in the CPB:

R31.0 - Hematuria
R31.9

Z12.6 - Encounter for screening for malignant neoplasm of bladder [diagnosis or screening in asymptomatic persons]

**Janus Kinase 2 (JAK2) mutations:**

CPT codes covered if selection criteria are met:

81270 - JAK2 (Janus kinase 2) (eg, myeloproliferative disorder) gene analysis, p.Val617Phe (V617F) variant [not covered for diagnostic assessment of myeloproliferative disorders in children; and quantitative assessment of JAK2-V617F allele burden subsequent to qualitative detection of JAK2-V617F]

ICD-10 codes covered if selection criteria are met:

C92.10 - Chronic myeloid leukemia, BCR/ABL-positive
C92.12

D45 - Polycythemia vera
D47.1 - Chronic myeloproliferative disease D47.3 Essential (hemorrhagic) thrombocytemia D47.4 Osteomyelofibrosis
D75.81 - Myelofibrosis

**BRAF mutation analysis:**

CPT codes covered if selection criteria are met:

81210 - BRAF (v-raf murine sarcoma viral oncogene homolog B1) (eg, colon cancer), gene analysis, V600E variant

ICD-10 codes covered if selection criteria are met:

C18.0 - Malignant neoplasm of colon, rectosigmoid junction, rectum, anus and anal canal
C43.0 - Melanoma of skin [for consideration of Vemurafenib, Dabrafenib and Trametinib]
C43.9
C49.4 - Malignant neoplasm of connective and soft tissue of abdomen [gastrointestinal stromal tumors]
C91.40 - Hairy cell leukemia
C91.42
D44.0 - Neoplasm of uncertain behavior of thyroid gland [indeterminate thyroid nodules]

**ICD-10 codes not covered if selection criteria are met:**
C34.00 - Malignant neoplasm of bronchus, and lung
C34.92
C73 - Malignant neoplasm of thyroid gland

**Assaying for loss of heterozygosity (LOH) on the long arm of chromosome 18 (18q) or deleted in colon cancer (DCC) protein (18q-LOH/DCC) for colorectal cancer:**
No specific code

**ICD-10 codes not covered for indications listed in the CPB:**
C18.0 - C20 Malignant neoplasm of colon, rectum, and rectosigmoid junction

**OvaCheck test:**
No specific code

**ICD-10 codes not covered for indications listed in the CPB:**
C56.1 - C56.9 Malignant neoplasm of ovary
Z12.73 Encounter for screening for malignant neoplasm of ovary

**Ovasure - No specific code:**

**Other CPT codes related to the CPB:**
82985 Glycated protein
83520 Immunoassay, analyte quantitative; not otherwise specified
84146 Prolactin
84305 Somatomedin
86304 Immunoassay for tumor antigen, quantitative; CA 125
Circulating cell-free nucleic acids - No specific code:

ICD-10 codes not covered for indications listed in the CPB:

C18.0 - C20 Malignant neoplasm of colon, rectosigmoid junction, and rectum

Circulating tumor cell (CTC) (e.g., CellSearch assay) & Circulating cell-free nucleic acids:

CPT codes not covered for indications listed in the CPB:

86152 Cell enumeration using immunologic selection and identification in fluid specimen (eg, circulating tumor cells in blood)
86153 physician interpretation and report, when required
88346 Immunofluorescence, per specimen; initial single antibody stain procedure
88361 Morphometric analysis, tumor immunohistochemistry (e.g., Her-2/neu, estrogen receptor/progesterone receptor), quantitative or semiquantitative, each antibody; using computer-assisted technology

ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):

C18.0 - C20 Malignant neoplasm of colon, rectosigmoid junction, and rectum
C43.0 - C43.9 Malignant melanoma of skin
C50.011 - C50.929 Malignant neoplasm of breast
C61 Secondary malignant neoplasm of breast

Cofilin (CFL1) - No specific code:

ICD-10 codes not covered for indications listed in the CPB:

C34.00 - Malignant neoplasm of bronchus and lung [non-small-cell lung cancer]
C34.92

ColonSentry - No specific code:

ICD-10 codes not covered for indications listed in the CPB:
Z12.11 - Encounter for screening for malignant neoplasm of colon and rectum

Decipher test (a RNA biomarkers assay) - No specific code:

ICD-10 codes not covered for indications listed in the CPB:
C61 Malignant neoplasm of prostate

Early CDT-Lung Test:

CPT codes not covered for indications listed in the CPB:
83520 Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise specified [as a screening for the early detection of lung cancer]

Galectin-3:

CPT codes not covered for indications listed in the CPB:
82777 Galectin-3

ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):
C61 Malignant neoplasm of prostate

Ki67:

CPT codes not covered for indications listed in the CPB:
88360 Morphometric analysis, tumor immunohistochemistry (eg, Her-2/neu, estrogen receptor/progesterone receptor), quantitative or semiquantitative, per specimen, each single antibody stain procedure; manual
88361 using computer-assisted technology

ICD-10 codes not covered for indications listed in the CPB:
C50.011 - Malignant neoplasm of breast
C50.929
C64.1 0 Malignant neoplasm of kidney, renal pelvis, and ureter
C66.9

Mammaprint:

HCPCS codes not covered for indications listed in the CPB:
S3854 Gene expression profiling panel for use in the management of breast cancer treatment
Mammostrat - No specific code:

**MLH1 tumor promoter hypermethylation:**

CPT codes not covered for indications listed in the CPB:

81288 MLH1 (mutl homolog 1, colon cancer, nonpolyposis type 2) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; promoter methylation analysis

ICD-10 codes not covered for indications listed in the CPB:

C54.1 Malignant neoplasm of endometrium

**Mucin 4 expression:**

CPT codes not covered for indications listed in the CPB:

88313 Group II, all other (eg, iron, trichrome), except immunocytochemistry and immunoperoxidase stains, including interpretation and report, each

ICD-10 codes not covered for indications listed in the CPB:

C18.0 - C20 Malignant neoplasm of colon, rectum and rectosigmoid junction

**Mucin 5AC (MUC5AC) - No specific code:**

ICD-10 codes not covered for indications listed in the CPB:

C22.1 Intrahepatic bile duct carcinoma

C24.0 - C24.9 Malignant neoplasm of other and unspecified parts of biliary tract

**Microarray-based gene expression profile testing:**

Other CPT codes related to the CPB:

81406 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)

**OVA1:**

CPT codes not covered for indications listed in the CPB:
Oncology (ovarian) biochemical assays of five proteins (apolipoprotein A-1, CA 125 II, follicle stimulating hormone, human epididymis protein 4, transferrin), utilizing serum, algorithm reported as a likelihood score

Oncology (ovarian), biochemical assays of five proteins (CA-125, apolipoprotein A1, beta-2 microglobulin, transferrin and pre-albumin), utilizing serum, algorithm reported as a risk score

p16 protein expression - No specific code:

ICD-10 codes not covered for indications listed in the CPB:
C00.0 - Malignant neoplasms of lip, oral cavity and pharynx
C14.8 [non-oropharyngeal squamous cell carcinoma]

Pathwork Tissue of Origin Test:

CPT codes not covered for indications listed in the CPB:
81504 Oncology (tissue of origin), microarray gene expression profiling of > 2000 genes, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as tissue similarity scores

PreOvar Test for the KRAS-variant [to determine ovarian cancer risk]:

CPT codes not covered for indications listed in the CPB:
83890 Molecular diagnostics; molecular isolation or extraction, each nucleic acid type (ie. DNA or RNA)
83896 Nucleic acid probe, each
83898 Amplification, target each nucleic acid sequence
83907 Lysis of cells prior to nucleic acid extraction (eg, stool specimens, paraffin embedded tissue), each specimen
83912 Interpretation and report

ICD-10 codes not covered for indications listed in the CPB:
C56.1 - Malignant neoplasm of ovary
C56.9
Z85.43 Personal history of malignant neoplasm of ovary

ProOnc Tumor Source Dx Test - No specific code:
ROMA:

CPT codes not covered for indications listed in the CPB:

81500 Oncology (ovarian), biochemical assays of two proteins (CA-125 and HE4), utilizing serum, with menopausal status, algorithm reported as a risk score
86304 Immunoassay for tumor antigen, quantitative; CA 125
86305 Human epididymis protein 4 (HE4)

ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):
C56.1 - Malignant neoplasm of ovary
C56.9

Rotterdam Signature 76-gene Panel:

HCPCS codes not covered for indications listed in the CPB:
S3854 Gene expression profiling panel for use in the management of breast cancer treatment

Serum amyloid A:

CPT codes not covered for indications listed in the CPB:

88342 Immunohistochemistry or immunocytochemistry, each separately identifiable antibody per block, cytologic preparation, or hematologic smear; first separately identifiable antibody per slide

Other CPT codes related to the CPB:

88341 - Immunohistochemistry or immunocytochemistry, per specimen
88344

ICD-10 codes not covered for indications listed in the CPB:
C54.0 - Malignant neoplasm of corpus uteri, isthmus and uterus
C54.8
Z85.42 Personal history of malignant neoplasm of uterus

Breast Cancer Gene Expression Ratio (HOXB13:IL17BR): HCPCS codes not covered for indications listed in the CPB:
S3854 Gene expression profiling panel for use in the management of breast cancer treatment

PAM50 ROR (Prosigna Breast Cancer Prognostic Gene Signature Assay):
**CPT codes covered if selection criteria are met:**

0008M Oncology (breast), MRNA analysis of 58 genes using hybrid capture, on formalin-fixed paraffin-embedded (FFPE) tissue, prognostic algorithm reported as a risk score [Prosigna]

**CPT codes not covered for indications listed in the CPB:**

81406 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) [when specified as the following]: PALB2 (partner and localizer of BRCA2) (eg, breast and pancreatic cancer), full gene sequence

**ICD-10 codes covered if selection criteria are met:**

C50.011 - Malignant neoplasm of breast
C50.929

**ICD-10 codes not covered for indications listed in the CPB:**

C61 Malignant neoplasm of prostate

**PTEN gene expression:**

**CPT codes covered if selection criteria are met:**

81321 - 81323 PTEN (phosphatase and tensin homolog) (eg, Cowden syndrome, PTEN hamartoma tumor syndrome) gene analysis

**ICD-10 codes covered for indications listed in the CPB:**

Q85.8 Other phakomatoses, not elsewhere classified [Cowden syndrome]

**ICD-10 codes not covered for indications listed in the CPB:**

C34.00 - Malignant neoplasm of bronchus and lung [non-small cell lung cancer]
C34.92

**GeneSearch Breast Lymph Node (BLN) assay - No specific code:**

**Thymidylate synthase - No specific code:**

No specific code

**Other CPT codes related to the CPB:**
88341 - Immunohistochemistry or immunocytochemistry, per specimen
88344
88360 - Morphometric analysis, tumor immunohistochemistry (eg, Her-2/neu, estrogen receptor/progesterone receptor), quantitative or semiquantitative, per specimen, each single antibody stain procedure; manual
88361 - using computer-assisted technology

Topographic genotyping (PathfinderTG) - No specific code:
Biomarker Translation (BT) - No specific code:

ICD-10 codes not covered for indications listed in the CPB:
C50.011 - Malignant neoplasm of breast
C50.929

HE4:

CPT codes not covered for indications listed in the CPB:
81500 - Oncology (ovarian), biochemical assays of two proteins (CA-125 and HE4), utilizing serum, with menopausal status, algorithm reported as a risk score
86305 - Human epididymis protein 4 (HE4)

Other CPT codes related to the CPB:
86316 - Immunoassay for tumor antigen; other antigen, quantitative (e.g., CA 50, 72-4, 549), each

ICD-10 codes not covered for indications listed in the CPB:
C54.1 - Malignant neoplasm of endometrium
C56.1 - Malignant neoplasm of ovary
C56.9
R19.00 - Intra-abdominal and pelvic swelling, mass, lump, unspecified site [not covered for evaluation of pelvic mass, including assistance in the determination of referral for surgery to a gynecologic oncologist or general surgery]
R19.07 - Generalized and other intra-abdominal and pelvic swelling, mass and lump [not covered for evaluation of pelvic mass, including assistance in the determination of referral for surgery to a gynecologic oncologist or general surgery]

**HERmark - No specific code:**

**ICD-10 codes not covered for indications listed in the CPB:**
- C50.011 - Malignant neoplasm of breast
- C50.929
- D05.00 - Carcinoma in situ of breast
- D05.92

**TargetPrint Gene Expression:**

**Other CPT codes related to the CPB:**
- 88360 Morphometric analysis, tumor immunohistochemistry (eg, Her-2/neu, estrogen receptor/progesterone receptor), quantitative or semiquantitative, per specimen, each single antibody stain procedure; manual
- 88361 using computer-assisted technology
- 88367 - Morphometric analysis, in situ hybridization
- 88377 (quantitative or semiquantitative)

**HCPCS codes not covered for indications listed in the CPB:**
- S3854 Gene expression profiling panel for use in the management of breast cancer treatment

**ICD-10 codes not covered for indications listed in the CPB:**
- C50.011 - Malignant neoplasm of breast
- C50.929

**TP53 - No specific code:**

**ICD-10 codes not covered for indications listed in the CPB:**
- C56.1 - Malignant neoplasm of ovary
- C56.9

**CK5, CK14, p63 and Racemase P504S:**

**Other CPT codes related to the CPB:**
88341 - Immunohistochemistry or immunocytochemistry, per specimen
88344 -

**ICD-10 codes not covered for indications listed in the CPB:**

C61 Malignant neoplasm of prostate  *EML4-ALK*

**ALK:**

**Other CPT codes related to the CPB:**

83891 Molecular diagnostics; isolation or extraction of highly purified nucleic acid, each nucleic acid type (ie, DNA or RNA)
83892 enzymatic digestion, each enzyme treatment
83894 separation by gel electrophoresis (eg, agarose, polyacrylamide), each nucleic acid preparation
83896 nucleic acid probe, each
83898 amplification, target, each nucleic acid sequence
83900 amplification, target, multiplex, first two nucleic acid sequence
83901 amplification, target, multiplex, each additional nucleic acid sequence beyond 2 (list separately in addition to code for primary procedure)
83902 reverse transcription
83904 mutation identification by sequencing, single segment, each segment
83907 lysis of cells prior to nucleic acid extraction (eg, stool specimens, paraffin embedded tissue), each nucleic acid preparation
83909 separation and identification by high resolution technique (eg, capillary electrophoresis), each nucleic acid preparation
83912 interpretation and report
88381 Microdissection (ie, sample preparation of microscopically identified target); manual

**ICD-10 codes not covered for indications listed in the CPB:**

C34.0 - Malignant neoplasm of bronchus and lung [non-small-cell lung cancer]
C34.92 -
Coloprint, CIMP, Line-1 hypomethylation and immune cells - No specific code:

ICD-10 codes not covered for indications listed in the CPB:
C18.0 - C20  Malignant neoplasm of colon, rectosigmoid junction and rectum

Des-gamma-carboxyl prothrombin (DCP):
CPT codes not covered for indications listed in the CPB:
83951  Oncoprotein; des-gamma-carboxy-prothrombin (DCP)

ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):
C22.0  Liver cell carcinoma
D01.5  Carcinoma in situ of liver and biliary system

5-hydroxyindoleacetic acid (5-HIAA):
CPT codes covered for indications listed in the CPB:
83497  Hydroxyindolacetic acid, 5-(HIAA)

ICD-10 codes covered if selection criteria are met:
C7A.00 - C7A.8  Malignant neuroendocrine tumors
C7A.8
D3A.00 - D3A.8  Benign neuroendocrine tumors
D3A.8

Beta-2 microglobulin (B2M):
CPT codes covered for indications listed in the CPB:
82232  Beta-2 microglobulin

ICD-10 codes covered if selection criteria are met:
C85.10 - C85.99  Other specified and unspecified types of non-Hodgkin lymphoma
C88.0  Waldenstrom macroglobulinemia
C90.00 - C90.02  Multiple myeloma
C90.02

CALCA (Calcitonin) expression:
CPT codes covered for indications listed in the CPB:
82308  Calcitonin

ICD-10 codes covered if selection criteria are met:
C73 Malignant neoplasm of thyroid
C76.0 Malignant neoplasm of head, face and neck

**CALB2 (Calretinin) expression:**

**CPT codes covered if selection criteria are met:**

88342  Immunohistochemistry or immunocytochemistry, each separately identifiable antibody per block, cytologic preparation, or hematologic smear; first separately identifiable antibody per slide

88341  each additional single antibody stain procedure (List separately in addition to code for primary procedure)

**ICD-10 codes covered if selection criteria are met:**

C34.00 - Malignant neoplasm of bronchus and lung  
C34.92  
C80.0 - Disseminated and other malignant neoplasm, 
C80.1  unspecified

**CHGA (Chromogranin A) expression:**

**CPT codes covered for indications listed in the CPB:**

86316  Immunoassay for tumor antigen; other antigen, quantitative (e.g., CA 50, 72-4, 549), each

**ICD-10 codes covered if selection criteria are met:**

C34.00 - Malignant neoplasm of bronchus and lung [non-small cell lung cancer]  
C4A.0 - Merkel cell carcinoma  
C4A.9  
C7A.00 - Malignant neuroendocrine tumors  
C7A.8  
C80.0 - Disseminated and other malignant neoplasm, 
C80.1  unspecified

D3A.00 - Benign neuroendocrine tumors  
D3A.8

**Beta human chorionic Gonadotropin (beta-hCG):**

**CPT codes covered for indications listed in the CPB:**

84704  Gonadotropin, chorionic (hCG); free beta chain

**ICD-10 codes covered if selection criteria are met:**
C37  Malignant neoplasm of thymus
C56.1 - Malignant neoplasm of ovary
C56.9
C62.00 - Malignant neoplasm of testis
C62.92
D07.39  Carcinoma in situ of other female genital organs
D07.69  Carcinoma in situ of other male genital organs [testis]
D15.0  Benign neoplasm of thymus
D27.0 - Benign neoplasm of ovary
D27.9
D29.20 - Benign neoplasm of testis
D29.22
N50.8  Other specified disorders of male genital organs
       [testicular mass]
R19.00  Intra-abdominal and pelvic swelling, mass, lump, 
       unspecified site
R19.07 -  Generalized and other intra-abdominal and pelvic 
R19.09  swelling, mass and lump
R22.2  Localized swelling, mass and lump, trunk

Isocitrate dehydrogenase 1 and 2 (IDH1, IDH2):

CPT codes covered for indications listed in the CPB:

83570  Isocitric dehydrogenase (IDH)

ICD-10 codes covered if selection criteria are met:

C40.00 -  Malignant neoplasm of bone and articular cartilage
C41.9   [chondrosarcoma]
C71.0 -  Malignant neoplasm of brain, spinal cord, cranial
C71.9   nerves and other parts of central nervous system
        [glioma] [glioblastoma]
C92.00 -  Acute myeloid leukemia (AML)
C92.02,
C92.40 -
C92.a2

INHA (Inhibin) expression:

CPT codes covered for indications listed in the CPB:
86336 Inhibin A

**ICD-10 codes covered if selection criteria are met:**

C56.1 - Malignant neoplasm of ovary
C56.9
D07.39 Carcinoma in situ of other female genital organs
D27.0 - Benign neoplasm of ovary
D27.9
R19.00 Intra-abdominal and pelvic swelling, mass, lump, unspecified site
R19.07 - Generalized and other intra-abdominal and pelvic swelling, mass and lump

**Lactate dehydrogenase (LDH):**

**CPT codes covered for indications listed in the CPB:**

83615 Lactate dehydrogenase (LD), (LDH)
83625 isoenzymes, separation and quantitation

**ICD-10 codes covered if selection criteria are met:**

C34.00 - Malignant neoplasm of bronchus and lung
C34.92
C40.00 - Malignant neoplasm of bone and articular cartilage
C41.9
C56.1 - Malignant neoplasm of ovary
C56.9
C62.00 - Malignant neoplasm of testis
C62.92
C64.1 - Malignant neoplasm of kidney and renal pelvis
C65.9
C85.10 - hodgkin's lymphoma
C85.99
C90.00 - Multiple myeloma
C90.02
C91.00 - Acute lymphoblastic leukemia (ALL)
C91.02
D02.20 - Carcinoma in situ of bronchus and lung
D07.39 Carcinoma in situ of other female genital organs
D07.69 Carcinoma in situ of other male genital organs [testis]
D14.30 - Benign neoplasm of bronchus and lung
D14.32
D16.0 - Benign neoplasm of bone and articular cartilage
D16.9
D27.0 - Benign neoplasm of ovary
D27.9
D29.20 - Benign neoplasm of testes
D29.22
D30.00 - Benign neoplasm of kidney and renal pelvis
D30.12
N28.89 Other specified disorders of kidney and ureter [kidney mass]
N50.8 Other specified disorders of male genital organs [testicular mass]
R19.00 Intra-abdominal and pelvic swelling, mass, lump, unspecified site
R19.07 - Generalized and other intra-abdominal and pelvic swelling, mass and lump
R19.09

**PDGFRB testing - No specific code:**

**ICD-10 codes covered if selection criteria are met:**
D46.0 - Myelodysplastic syndromes (MDS)
D46.9
D48.5 Neoplasm of uncertain behavior of skin [dermatofibrosarcoma]

*Quest Diagnostic Thyroid Cancer Mutation Panel:*

**CPT codes covered for indications listed in the CPB:**
81445 Targeted genomic sequence analysis panel, solid organ neoplasm, DNA analysis, and RNA analysis when performed, 5-50 genes (eg, ALK, BRAF, CDKN2A, EGFR, ERBB2, KIT, KRAS, NRAS, MET, PDGFRA, PDGFRB, PGR, PIK3CA, PTEN, RET), interrogation for sequence variants and copy number variants or rearrangements, if performed
ICD-10 codes covered if selection criteria are met:

E04.0 - E04.9 Other nontoxic goiter [thyroid nodules] [not covered for repeat testing of indeterminate thyroid nodules]

RUNX1 mutation:

CPT codes covered for indications listed in the CPB:

81401  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)

ICD-10 codes covered if selection criteria are met:

D46.0 - D46.9 Myelodysplastic syndromes (MDS)
D46.9

Thymidine kinase:

CPT codes covered for indications listed in the CPB:

81405  Molecular pathology procedure, Level 6 (eg, analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis)

ICD-10 codes covered if selection criteria are met:

C91.10 - C91.12, C91.90 - C91.91 Chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL)
C91.91

Thyroglobulin antibody:

CPT codes covered for indications listed in the CPB:

86800  Thyroglobulin antibody

ICD-10 codes covered if selection criteria are met:

C73  Malignant neoplasm of thyroid gland
D09.3  Carcinoma in situ of thyroid and other endocrine glands
D34  Benign neoplasm of thyroid gland

Thyroglobulin (TG) expression:

CPT codes covered for indications listed in the CPB:
Thyroglobulin

**ICD-10 codes covered if selection criteria are met:**

C73  Malignant neoplasm of thyroid gland  
C76  Malignant neoplasm of head, face and neck  
C80.0 - Disseminated and other malignant neoplasm, unspecified  
D09.3  Carcinoma in situ of thyroid and other endocrine glands  
D34  Benign neoplasm of thyroid gland

**Thyroid transcription factor-1 (TTF-1):**

**CPT codes covered if selection criteria are met:**

88342  Immunohistochemistry or immunocytochemistry, each separately identifiable antibody per block, cytologic preparation, or hematologic smear; first separately identifiable antibody per slide  
88341  each additional single antibody stain procedure (List separately in addition to code for primary procedure)

**ICD-10 codes covered if selection criteria are met:**

C34.00 - Malignant neoplasm of bronchus and lung  
C34.92  
C7A.00 - Malignant neuroendocrine tumors  
C7A.8  
D02.20 - Carcinoma in situ of bronchus and lung  
D02.22  
D14.30 - Benign neoplasm of bronchus and lung  
D14.32  
D3A.00 - Benign neuroendocrine tumors  
D3A.8

**WT-1 gene expression - No specific code:**

**ICD-10 codes covered if selection criteria are met:**

C34.00 - Malignant neoplasm of bronchus and lung [non-small-cell lung cancer]  
C34.92  
C80.0 - Disseminated and other malignant neoplasm, unspecified  
C80.1
Carcinoembryonic antigen cell adhesion molecule 6 (CEACAM6)
(e.g., Benign Diagnostics Risk Test) - No specific code:

ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):

N62 Hypertrophy of breast [breast atypical hyperplasia]

CDX2:

CPT codes not covered for indications listed in the CPB:

88342 Immunohistochemistry or immunocytochemistry, each separately identifiable antibody per block, cytologic preparation, or hematologic smear; first separately identifiable antibody per slide

88341 each additional single antibody stain procedure (List separately in addition to code for primary procedure)

ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):

C18.0 - Malignant neoplasm of colon
C18.9
D01.0 Carcinoma in situ of colon
D12.0 - Benign neoplasm of colon
D12.9

CxBladder test - No specific code:

ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):

C67.0 - Malignant neoplasm of bladder
C67.9

4Kscore:

CPT codes not covered for indications listed in the CPB:

81539 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

Fibrinogen degradation products (FDP) test (e.g., DR-70 or Onko-Sure) - No specific code:
ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):

- C18.0 - C20  Malignant neoplasm of colon, rectosigmoid junction, and rectum

**HMGB1 and RAGE - No specific code:**

ICD-10 codes not covered for indications listed in the CPB:

- C43.0 - C44.99  Melanoma and other malignant neoplasms of skin
- C44.99

**IHC4 (e.g., NexCourse IHC4) - No specific code:**

ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):

- C50.011 - C50.929  Malignant neoplasm of breast
- C50.929

**Lectin-reactive alpha-fetoprotein (AFP-L3):**

CPT codes not covered for indications listed in the CPB:

- 82107  Alpha-fetoprotein (AFP); AFP-L3 fraction isoform and total AFP (including ratio)

ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):

- C22.0, C22.2  Malignant neoplasm of liver
- C22.9

**Liquid biopsy (e.g., CancerIntercept, GeneStrat):**

CPT codes not covered for indications listed in the CPB:

- 86152  Cell enumeration using immunologic selection and identification in fluid specimen (eg, circulating tumor cells in blood)
- 86153  physician interpretation and report, when required

ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):

- C18.0 - C20  Malignant neoplasm of colon, rectosigmoid junction, and rectum
- C34.00 - C34.92  Malignant neoplasm of bronchus and lung
C43.0 - Malignant melanoma of skin
C43.9
C50.011 - Malignant neoplasm of breast
C50.929
C56.1 - Malignant neoplasm of ovary
C56.9

*Long non-coding RNA - No specific code:*

ICD-10 codes not covered for indications listed in the CPB:

C23 Malignant neoplasm of gallbladder

*Mass spectrometry-based proteomic profiling (e.g., Xpresys Lung):*

CPT codes not covered for indications listed in the CPB:

81538 Oncology (lung), mass spectrometric 8-protein signature, including amyloid A, utilizing serum, prognostic and predictive algorithm reported as good versus poor overall survival

ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):

R91.8 Other nonspecific abnormal finding of lung field [indeterminate pulmonary nodules]

*OncoVantage:*

CPT codes not covered for indications listed in the CPB:

81445 Targeted genomic sequence analysis panel, solid organ neoplasm, DNA analysis, and RNA analysis when performed, 5-50 genes (eg, ALK, BRAF, CDKN2A, EGFR, ERBB2, KIT, KRAS, NRAS, MET, PDGFRA, PDGFRB, PGR, PIK3CA, PTEN, RET), interrogation for sequence variants and copy number variants or rearrangements, if performed

*Experimental and investigational circulating tumor markers: CPT codes not covered for indications listed in the CPB: 0006M*

Oncology (hepatic), MRNA expression levels of 161 genes, utilizing fresh hepatocellular carcinoma tumor tissue, with alpha-fetoprotein level, algorithm reported as a risk classifier [Heprodx]
0007M Oncology (gastrointestinal neuroendocrine tumors), real-time PCR expression analysis of 51 genes, utilizing whole peripheral blood, algorithm reported as a nomogram of tumor disease index [Netest]

81218 CEBPA (CCAAT/enhancer binding protein [C/EBP], alpha) (eg, acute myeloid leukemia), gene analysis, full gene sequence

81455 Targeted genomic sequence analysis panel, solid organ or hematolymphoid neoplasm, DNA analysis, and RNA analysis when performed, 51 or greater genes (eg, ALK, BRAF, CDKN2A, CEBPA, DNMT3A, EGFR, ERBB2, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, KRAS, MLL, NPM1, NRAS, MET, NOTCH1, PDGFRA, PDGFRB, PGR, PIK3CA, PTEN, RET), interrogation for sequence variants and copy number variants or rearrangements, if performed

81540 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 a predicted main cancer type and subtype

81545 Oncology (thyroid), gene expression analysis of 142 genes, utilizing fine needle aspirate, algorithm reported as a categorical result (eg, benign or suspicious)

82387 Cathepsin-D

84275 Sialic acid

86316 Immunoassay for tumor antigen; other antigen, quantitative (e.g., CA 50, 72-4, 549), each

88342 Immunohistochemistry or immunocytochemistry, each separately identifiable antibody per block, cytologic preparation, or hematologic smear; first separately identifiable antibody per slide [Cyclin E (fragments or whole length)]

There are no specific codes for the circulating tumor markers listed below:
anti-VEGF antibody bevacizumab; BluePrint molecular subtyping profile for breast cancer; Catalytic subunit alpha polypeptide gene (PIK3CA); C-Met expression; DCIS Recurrence Score; Glutathione-S-transferase P1 (GSTP1); Percepta Bronchial Genomic Classifier; Phosphatidylinositol-4,5-bisphosphonate 3-kinase; Proveri prostate cancer assay (PPCA); Ribonucleotide reductase subunit M1 (RRM1); ROS1 re-arrangements; Previstage GCC; Prostate core mitotic test; Prolaris; UroCor cytology assay (DD23 and P53); Breast cancer index; PAM50 ROR (Prosigna Breast Cancer Prognostic Gene Signature Assay); BioSpeciFx; Guardant 360

The above policy is based on the following references:


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AETNA BETTER HEALTH® OF PENNSYLVANIA

Amendment to
Aetna Clinical Policy Bulletin Number: 0352 Tumor Markers

There are no amendments for Medicaid.