



Cigna Medical Coverage Policy

Subject **Circulating Tumor Cells Testing**

Effective Date 2/15/2014
Next Review Date 2/15/2015
Coverage Policy Number 0262

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Coverage Policy

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General Background

Circulating tumor cells (CTCs) have been found in the circulation of patients with various forms of metastatic carcinomas. The detection of circulating tumor cells has been proposed as a method to monitor progression and assess response to treatment. The detection of tumor cells may have clinical utility in risk stratification in early breast cancer, in early detection of relapse and in monitoring the response to treatment (Ring, et al., 2005). The circulating cells appear to have characteristics of tumor cells and may be identified in the peripheral blood of patients with cancer.

The techniques that have been used to detect CTCs include cytometric and nucleic acid based approaches. The cytometric approaches use immunocytochemical methods to identify and characterize the individual tumor cells. Nucleic acid based approaches detect the DNA and RNA sequences that are differentially expressed in tumor cells and normal blood components (Ring, et al., 2004). The first studies of circulating tumor cells involved cases of breast cancer. Since that time, research is also being conducted that examines the detection of CTC in different cancers of epithelial origin, including colon and prostate cancers.

It has been noted in the literature that, while pilot studies suggest that the identification of circulating cells may have a role in risk stratification and monitoring responses to treatment, larger longitudinal studies with standard techniques in clearly-defined populations of patients are needed to establish the clinical significance of circulating breast cancer cells (Ring, et al., 2004).

Currently effectiveness of treatment and disease progression is monitored by various tests which may include imaging tests (e.g., bone scan, radiograph, magnetic resonance imaging [MRI] and computed tomography [CT]) and/or tumor markers. Tumor markers are a less complicated means of monitoring response to treatment than imaging tests (American Cancer Society [ACS], 2009). If a tumor marker level in the blood goes down, that is almost always a sign that treatment is effective. Increasing levels of markers may indicate a need for more aggressive therapy. One exception is if the cancer is very sensitive to a particular chemotherapy treatment. The chemotherapy can cause many cancer cells to die rapidly and release large amounts of the marker, which will cause the level of the marker in the blood to rise temporarily. Other methods of monitoring treatment progress and metastatic disease progression are currently being developed and researched.

There are several methods of detecting circulating tumor cells which are in various stages of research and development. The low level of concentration of malignant epithelial cells in blood samples, approximately one in 10^6 to 10^7 total nucleated cells makes them difficult to detect (Ross, et al., 2009). Detection and enumeration of CTCs has been attempted with several methods including: polymerase chain reaction (PCR), flow cytometry, image-based immunologic approaches, immunomagnetic techniques and microchip technology (Allan, et al., 2010).

The CellSearch System™ (Veridex LLC, Warren, NJ) was developed for the purpose of detecting circulating tumor cells (CTCs) in whole blood. The CellSearch system involves a technique of mixing a blood sample with iron particles coated with an antibody that attaches to epithelial cells. The epithelial cells are then distinguished from leukocytes by antibodies that have been tagged with a fluorescent dye so that the cancer cells can be easily distinguished and counted. Since epithelial cells are not usually found in the blood, these cells are likely cancerous cells from the tumor.

AdnaTest BreastCancer® (AdnaGen AG, Hanover, Germany; OncoVista, Inc., San Antonio, TX) was developed for the detection and molecular analysis of circulating tumor cells. According to the vendor website, the test is currently marketed in Europe and plans are underway to obtain FDA approval. This system utilizes reverse transcriptase-polymerase chain reaction (RT-PCR) to detect circulating tumor cells. The test features a CTC-enrichment procedure that utilizes a proprietary mixture of immunomagnetic bead coated with one of three antibodies to epithelial surface antigens. The number of CTCs is then indirectly determined by a semiquantitative RT-PCR method using probes for three epithelial cell-associated mRNAs: MUC1, HER2, and the surface glycoprotein GA 733-2 (Ross, et al., 2009).

The OncoQuick™ (Greiner Bio-One, Inc., Longwood, FL) is another testing system that has been developed to detect circulating tumor cells. This system is an enhanced density gradient system that combines density gradient centrifugation and the immuno-based techniques. This system is not approved by the U.S. Food and Drug Administration's (FDA) and is available only for research purposes.

U.S. Food and Drug Administration (FDA)

In January 2004, the CellSearch Epithelial Cell Kit/CellSpotter Analyzer was cleared for marketing through the FDA's 510(k) process as a class II device (FDA, 2004). The intended use is for the enumeration of CTCs of epithelial origin in whole blood. In June 2004, the CellSearch Epithelial Control Kit was approved for use as an assayed control. In March 2005, the CellSearch Circulating Tumor Cell Kit, which incorporated modifications made to the prior device, was approved. In October 2005, a modification to the CellSearch Circulating Tumor Cell Kit was made by the FDA with no change in the intended use of the test.

In December 2006, a modification was approved by the FDA for the test to be used as an aid in the monitoring of patients with metastatic breast cancer. The FDA documentation notes that there is no direct risk to the patient from the use of this device. However, a false-negative test could lead to undetected progressive disease and failure to treat the patient accordingly, while a false-positive test could lead to unnecessary treatment.

In 2007, the FDA cleared the device for expanded the indications for use to include metastatic colon cancer (FDA, 2007). In 2008, the FDA granted clearance for an expanded indication of the device to include use in metastatic prostate cancer (FDA, 2008).

Literature Review for Circulating Tumor Cells in Breast Cancer

Lucci et al. (2012) conducted a prospective study to establish if CTCs predicted worse outcome in patients with non-metastatic breast cancer. Data was collected for CTCs at the time of definitive surgery from chemo-naive patients with stage 1–3 breast cancer (n=302). CellSearch System was used to measure CTCs. Findings of CTC were correlated with standard tumor characteristics, including tumor size and grade; estrogen and progesterone receptor and human epidermal growth factor receptor 2 (HER2) status; and axillary lymph node status. Outcomes assessed at a median follow-up of 35 months. Circulating tumor cells were identified in 73 (24%) patients. Detection of one or more circulating tumor cells predicted both decreased progression-free survival (log-rank p=0.005; hazard ratio [HR] 4.62; 95% CI 1.79–11.9) and overall survival (log-rank p=0.01; HR 4.04, 1.28–12.8). The authors interpreted the results to indicate that the presence of one or more circulating tumor cells predicted early recurrence and decreased overall survival in chemo-naive patients with non-metastatic breast cancer. Further studies are needed to demonstrate the clinical utility of CTC in patients with non-metastatic breast cancer.

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

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

Pierga et al (2011) conducted a prospective observational study that examined the correlation with tumor markers (CA 15-3, carcinoembryonic antigen and lactate dehydrogenase). CTCs were counted with CellSearch at baseline, prior to cycle 2 (C2) and cycle 3 or 4 (C3 or C4) in 267 metastatic breast cancer patients on first-line chemotherapy with and without targeted therapy. The baseline CTC detection rate was 65% with ≥ 1 CTC/7.5 ml threshold and 44% with ≥ 5 CTC/7.5 ml and was independent of subtypes (luminal, triple negative, human epithelial growth factor receptor 2 (HER2) positive). CTCs were found to correlate with tumor markers, bone and liver involvement, tumor burden and performance status. CTC detection ≥ 1 CTC/7.5 ml was a strong prognostic factor for progression-free survival (PFS) (p<0.0001). Threshold of CTC ≥ 5 was statistically significant for PFS and overall survival (OS) (p=0.03 on multivariate analysis). Among patients with ≥ 5 CTC/7.5 ml at baseline, 50% had <5 CTC/7.5 ml when measured at C2. The changes were associated with both PFS and OS (p<0.0001). All patients that received anti-HER2 therapy had <5 CTC/7.5 ml after three cycles of treatment. While there appears to be a correlation with tumor markers regarding OS and PFS the study does not demonstrate clinical utility of CTC testing.

Guiliano et al. (2011) retrospectively assessed the prognostic impact of CTCs according to different first-line systemic treatments, and the potential predictive value of CTCs in 235 metastatic breast cancer (MBC) patients. Progression-free survival and overall survival were compared with the log-rank test between groups, according to CTC count (<5 vs ≥5) and the type of systemic therapy. Further exploration was performed of the predictive value of baseline CTCs in patients receiving different treatments. At a median follow-up of 18 months, the CTC count was confirmed to be a strong prognostic marker in the overall population (median progression-free survival 12.0 and 7.0 months for patients with CTC< 5 and ≥5, respectively; $p<0.001$). In contrast, in patients with human epidermal growth factor receptor-2-overexpressed/amplified tumors receiving trastuzumab or lapatinib, the baseline CTC count was not prognostic (median progression-free survival 14.5 months for patients with CTC<5 and 16.1 months for those with CTC≥5; $p=0.947$). In addition, in patients with human epidermal growth factor receptor-2 normal tumors, a baseline CTC count ≥ 5 identified individuals who derived benefit from more aggressive treatments, including combination chemotherapy and chemotherapy plus bevacizumab. This study provides preliminary indication that type of therapy may have an effect on CTC levels. Limitations of the study included lack of randomized comparisons, and the limited number of patients. Further prospective randomized studies are needed to confirm these results and demonstrate clinical utility.

Bidard et al. (2010b) reported on a prospective, observational study that investigated whether CTCs and circulating endothelial cells (CECs) can predict clinical outcome of first-line chemotherapy combined with bevacizumab in 67 metastatic breast cancer patients. CTC and CEC counts (with CellSearch system) at baseline and changes after two cycles of treatment were correlated with time to progression (TtP). Findings indicated that CTC and CEC levels were not correlated. At baseline, the CTC positivity was a significant prognostic marker for TtP at a threshold of 3 CTC/7.5 ml ($p<0.05$) but not at 5 CTC/7.5 ml ($p=0.09$). The baseline CEC levels (median 17 CEC/4 ml, range 1–769) were associated with age ≥45 years ($p=0.01$), elevated lactate dehydrogenase ($p<0.01$) and not with TtP at any threshold. The changes of CTC count during treatment were not found to be a surrogate of TtP, with any of the model tested (threshold based or relative decrease in percent). Although, an increase in CEC count was found to be associated with improved TtP, at the threshold of 20 CEC/4 ml ($p<0.01$). The authors concluded that Bevacizumab combined with first-line chemotherapy may modify the predictive value of CTC during treatment possibly due to impaired tumor cells intravasation through vessels endothelium. This study indicates that the prognostic value of CTC may be affected by the treatment provided. The findings need to be confirmed in larger randomized study.

Bidard et al. (2010a) reported on clinical outcomes of 115 nonmetastatic breast cancer patients according to CTC detection. At baseline, 23% of patients were CTC positive, but only 10% had >1 CTC/7.5 ml of blood. After a median follow-up of 36 months, CTC detection before chemotherapy was an independent prognostic factor for both distant metastasis-free survival (DMFS) ($p=0.01$, relative risk [RR]=5.0, 95% confidence interval [CI] 1.4–17) and overall survival (OS) ($p=0.007$, RR=9, 95% CI 1.8–45). The detection of CTC after chemotherapy was found to be of less significance ($p=0.07$ and 0.09, respectively). The authors conclude that detection of ≥ 1 CTC/7.5 ml before neoadjuvant chemotherapy can accurately predict OS. Further trials are needed to confirm the results.

Sieuwerts et al. (2009) examined whether the five subtypes of breast cancer cells that have been defined by global gene expression profiling—normal-like, basal, HER2-positive, and luminal A and B—were identified with CellSearch test. Global gene expression profiling was used to determine the subtypes of a well-defined panel of 34 human breast cancer cell lines (15 luminal, nine normal-like, five basal-like, and five Her2-positive). Cells from each of these cell lines were mixed with 7.5 ml of blood from a single healthy human donor, and the mixtures were subjected to the CellSearch test to isolate the breast cancer cells. It was found that the CellSearch isolation method, which uses EpCAM on the surface of circulating tumor cells for cell isolation, did not recognize normal-like breast cancer cells, which in general have aggressive features.

Liu et al. (2009) conducted on a prospective study that examined the correlation of CTCs with radiographic findings for disease progression. Serial CTC levels were obtained in patients ($n=68$) that were starting a new treatment regimen for progressive, radiographically measurable metastatic breast cancer. Blood was collected at baseline and three to four week intervals and radiographic studies were performed in nine to twelve week intervals. Median follow-up was 13.3 months. Patients who had five or more CTCs had 6.3 times the odds of radiographic disease progression when compared with patients who had less than five CTCs. Shorter progression-free survival was observed for patients with five or more CTCs at three to five weeks and at seven to nine weeks after the start of treatment. The CTC result was statistically significantly associated with disease progression for all patients ($p<.001$). The association was noted to remain strong in patients treated with either

chemotherapy or endocrine therapy. Potential limitations of the study include that the study included patients receiving various lines and types of therapy. The subgroup analysis for CTC-imaging correlation was performed by including biologic agents with either chemotherapy or endocrine therapy—it was noted that each group was too small to be analyzed alone.

Dawood et al. (2008) reported on a study with the purpose of assessing the prognostic value of baseline CTCs in a cohort of patients with newly diagnosed metastatic breast cancer. The retrospective study included 185 patients with newly diagnosed metastatic breast cancer evaluated between 2001 and 2007. Circulating tumor cells (CTCs) were isolated and enumerated with the CellSearch system before the patients started first-line treatment. Overall survival was calculated from the date of CTC measurement, estimated by the Kaplan-Meier product limit method, and compared between groups with the log-rank test. The association between CTC levels and overall survival were determined after controlling for other prognostic factors with Cox proportional hazards models. Fifty-six (30.3%) of the patients presented with de novo metastatic disease, and 129 (69.7%) patients presented with newly recurrent breast cancer. A total of 114 patients (61.6%) had CTC < 5, and 71 (38.4%) had CTC ≥ 5. The median overall survival was 28.3 months and 15 months ($p < .0001$) for patients with CTC < 5 and CTC ≥ 5, respectively. Superior survival among patients with CTC < 5 was observed regardless of hormone receptor and HER-2/neu status, site of first metastases, or whether the patient had recurrent or de novo metastatic disease. In the multivariate model, patients with CTC ≥ 5 had a hazards ratio of death of 3.64 (95% CI, 2.11-6.30) as compared with patients with CTC < 5.

Slade et al. (2008) conducted a study to determine whether primary breast cancer patients demonstrated evidence of CTCs during follow-up as an alternative to monitoring disseminated bone marrow tumor cells (DTCs) by immunocytochemistry and reverse transcriptase (RT)-PCR for the detection of micrometastases. The study involved two cohorts of primary breast cancer patients who were at low (group II; $n=18$) risk or high (group III; $n=33$) risk of relapse and who were being followed-up after primary treatment. Each cohort was tested for CTCs using the CellSearch system on one to seven occasions and for DTCs by immunocytochemistry and RT-PCR on one to two occasions over a period of two years. Patients with confirmed metastatic disease (group IV; $n=12$) and 21 control healthy volunteers for CTCs (group I) were also examined. All group I samples were negative for CTCs. Seven out of 18 (39%) in group II patients and 23 out of 33 (70%) of group III patients were positive for CTCs ($p=0.042$). When considering samples with greater than one cell as positive, then two out of 18 (11%) of group II patients were positive as compared with 10 out of 33 (30%) in group III ($p=0.174$). In the case of DTCs, one of 13 (8%) in group II patients were positive as compared with 19 out of 27 (70%) in group III ($p < 0.001$). Ten out of 33 (30%) patients in group III demonstrated no evidence of CTCs in all tests over the period of testing as compared with 11 out of 18 (61%) in group II ($p=0.033$). It appears that a significant proportion of poor prognosis primary breast cancer patients (group III) have evidence of CTCs on follow-up. Many of these patients also have evidence of DTCs, which are more often found in patients who were lymph node positive. As repeat sampling of peripheral blood is more acceptable to patients, the measurement of CTCs appears to warrant further investigation since it enables blood samples to be taken more frequently.

Nole et al. (2007) conducted a prospective study to evaluate the prognostic significance of CTCs detection in advanced breast cancer patients. The study included 80 patients with inclusion criteria: women with histological diagnosis of breast cancer, evidence of metastatic disease from imaging studies, starting a new line of therapy and/or treated for the advanced disease with a maximum two lines of therapy. The CellSearch system was used to test for circulating tumor cell levels before starting a new treatment and after four, eight weeks and the first clinical evaluation and every two months thereafter. At baseline, 49 patients were found to have ≥ 5 CTCs. The baseline number of CTCs were associated with progression-free survival (hazard ratio [HR] 2.5; 95% confidence interval [CI] 1.2–5.4). The risk of progression for patients with CTCs ≥ 5 at the last available blood draw was five times the risk of patients with 0–4 CTCs at the same time point (HR 5.3; 95% CI 2.8–10.4). At the last available blood draw, patients with rising or persistent CTCs ≥ 5 demonstrated a statistically significant higher risk of progression with respect to patients with CTCs < 5 at both blood draws (HR 6.4; 95% CI 2.8–14.6). The authors noted that these results indicate that elevated CTCs levels measured at any time in the clinical course of a patient with metastatic breast cancer predict an imminent progression and that this analysis represents an additional step in the process of validating this method. There are still unanswered questions regarding the treatment of a patient with low or high levels of CTCs in breast cancer.

Cristofanilli et al. (2007) reported on a retrospective analysis of 151 patients with metastatic breast cancer to compare the prognostic significance of CTCs with clinical and laboratory measures of tumor burden and phenotypic subtype of disease. Of the 151 patients, 32 were registered in the 2004 Cristofanilli study

(Cristofanilli, et al., 2004). The remaining 119 were a new cohort of patients who had CTCs measured as part of the laboratory evaluation at the time of staging of their metastatic disease and before starting a new treatment. The CTCs were isolated and enumerated in whole blood using the CellSearch System. The overall survival was evaluated according to the level of CTCs (negative: < 5 CTCs per 7.5 ml of blood; positive: ≥ 5 CTCs per 7.5 ml of blood), Swenerton score, CA 27.29 level, age (< 50 years vs. ≥ 50 years), hormone receptor status, and HER2 status, metastatic site, and type and line of therapy. The median age of patients was 53 (range of 24–88 years) and 44% of the patients had > 5 CTCs. The median overall survival for negative versus positive CTCs were 29.3 months and 13.5 months, respectively ($p < 0.0001$). Ninety patients (60%) had HR-positive disease, and 35 patients (23%) had HER2 amplification. Eighty-four patients (56%) had < 5 CTCs and were classified as negative for CTCs. Sixty-two (44%) patients had > 5 CTCs and were considered positive. The abnormal CA 27.29 levels were detectable in 98 patients (66%), and 47 patients (31%) had Swenerton scores > 20. When patients negative for CTCs were compared to patients who were positive, they tended to have abnormal CA 27.29 serum values more frequently. In addition, it was noted that there were trends of patients positive for CTCs having higher Swenerton scores and HER2 –negative disease with increased frequency. The median follow-up was 12.7 months (range of 0.2–66.3 months). With the multivariable Cox model, the detection of ≥ 5 CTCs demonstrated the highest hazard ratio with 2.2 times the risk of death ($p = 0.003$). The results indicated that the prognostic value was independent of measure of tumor burden and type and line of therapy and phenotypic subtype of the disease.

Budd et al. (2006) published a report that compared the testing of circulating tumor cells (CTCs) to radiology for prediction of overall survival in patients with metastatic breast cancer. This report involved a subset of the Cristofanilli study (Cristofanilli, et al., 2004). One hundred and thirty-eight of the 177 patients enrolled in the trial had imaging studies performed at baseline and at a median of 10 weeks after the initiation of therapy. All scans were reviewed by two independent radiologists to determine radiologic response. Circulating tumor cell counts were determined at approximately four weeks after initiation of therapy. Specimens were analyzed at one of seven laboratories and then reviewed by a central laboratory. The inter-reader reliability for the radiologic responses were 15.2% and 0.7% for circulating tumor cell counts. The median overall survival of 13 (9%) patients with radiologic nonprogression and greater than five CTCs were significantly shorter than that of the 83 (60%) of patients with radiologic nonprogression and less than five circulating tumor cells. The median overall survival of the 20 (14%) patients with radiologic progression and less than five CTCs was significantly longer than the 22 (16%) patients with greater than five CTCs who showed progression by radiology (19.9 as compared to 6.4 months).

Hayes et al. (2006) published additional follow-up data and evaluation of circulating tumor cell levels at subsequent visits for the 177 patients involved in the initial 2004 Cristofanilli study (Cristofanilli, et al., 2004). Serial blood specimens were collected monthly for a period of up to six months. Reassessment of disease status was conducted every nine to 14 weeks. Nine patients died and five patients withdrew from the study after the baseline blood sample. Of the remaining 163 patients with follow-up disease assessment, 26 patients had partial response to therapy, 82 had stable disease, and 55 had progressive disease at the time of first follow-up. None of the patients had a complete response to therapy at any time during the study. Progression-free survival and overall survival times were calculated from the dates of each follow-up blood draw. The median progression-free survival times for patients with less than five CTCs from each of the five blood draw time points were 7.0, 6.1, 5.6, 7.0, and 6.0 months, respectively. For patients with five or greater circulating tumor cells, the median progression-free survival from these same time points was significantly shorter: 2.7, 1.3, 1.4, 3.0, and 3.6 months, respectively. Median overall survival for patients with less than five CTCs from the five blood draw time points was all greater than 18.5 months. For patients with five or greater circulating tumor cells, median overall survival from these same time points was significantly shorter: 10.9, 6.3, 6.3, 6.6, and 6.7 months, respectively. Median progression-free survival and overall survival times at baseline and up to nine to 14 weeks after the initiation of therapy were noted to be statistically significantly different.

In 2005, Cristofanilli et al. published an analysis of a subset of the 2004 Cristofanilli study (Cristofanilli, et al., 2004; Cristofanilli, et al., 2005). This analysis focused on 83 patients with newly diagnosed measurable metastatic breast cancer who were about to start their first line of systemic therapy. The purpose of this analysis was to investigate whether the presence of CTCs predicts treatment efficacy, progression-free survival, and in patients with newly diagnosed metastatic breast cancer who were about to start first-line therapy. The mean follow-up time was a median of 12.2 months. Forty-three patients (52%) had greater than five circulating tumor cells (CTCs) at baseline. The median progression-free survival was 7.2 months, and the median overall survival was more than 18 months. Patients with five CTCs were noted to have a worse prognosis than patients with

less than five CTCs at baseline. It is noted that the subset analysis is not statistically significant because of the small number of patients, and the need for future investigations using CTCs may be indicated.

Allard et al. (2004) conducted a study to determine the accuracy of the CellSearch system and evaluate the number of CTCs in samples of blood from healthy subjects, patients with nonmalignant disease, and a variety of metastatic carcinomas. Samples from normal subjects were spiked with tumor cells, and the samples were tested with the CellSearch system. The average percentage of cells recovered was greater than 85%, with greater variation as the number of cells per sample decreased. The test was found to have 99.7% specificity. This was determined by comparing blood samples from women with and without breast disease for detection of cells. None of the healthy women, and only one woman with a nonmalignant disease, were found to have elevated circulating tumor cells. Circulating tumor cell counts were conducted on a total of 2183 blood samples from 964 patients with various metastatic carcinomas; however, the sensitivity and specificity of the test in cancers other than breast cancer were not documented.

Kahn et al. (2004) reported on a study that utilized a direct visualization assay to correlate the number of CTCs with disease stage and progression. The CTCs were enriched from the nucleated cell fraction by filtration and enumerated visually following immunostaining with anti-cytokeratin 8 (CK8) antibody CAM 5.2. No CTCs were detected in the control subjects (n=20). In 131 breast cancer patients, a higher incidence of CTCs occurred in patients with distant metastases (36/51 or 71%) than those with node-positive (17/36 or 47%) or node-negative (17/44 or 39%). The authors concluded that the results support the concept that CTCs can be detected and enumerated in peripheral blood and that this minimally invasive assay merits further evaluation as a potential prognostic indicator and marker of disease progression. Wong et al. (2006) reported follow-up data on the group from the Kahn study. In this report, the time to progression and overall survival were defined as interval from the first blood sampling to first documented disease progression or death. The follow-up data is available for 123 patients. In early disease, median CTCs distinguished patients with shorter time to progression. In metastatic disease, median CTCs optimally identified patients with shorter time to progression. It was noted that there was no relationship between circulating tumor cell level, and overall survival was found in this subgroup. The authors concluded that median circulating tumor cell level determined in the course of treatment predicts time to progression in metastatic breast cancer. In early breast cancer, an association was found between circulating tumor cell level and time to progression, although this did not reach statistical significance. It is also noted that further studies with predetermined timing and interval of blood sampling in relation to treatment are warranted and will better define the prognostic value of this method of circulating tumor cell enumeration and allow comparisons between different schedules of blood sampling. In addition, further research is needed regarding the potential ability to determine prognosis in early breast cancer based on circulating tumor cell level at the end of adjuvant therapy.

Cristofanilli et al. (2004) conducted a prospective study of 177 women with metastatic breast cancer compared to 345 women without breast cancer to confirm the correlation between the level of detection of peripheral-blood tumor cells and presence of metastatic breast disease. The breast cancer patients were tested prior to receiving breast cancer treatment and at the first follow-up after commencing treatment. The first 102 women with breast cancer were used as a training set to determine the number of circulating tumor cells (CTCs) that correlate with poor prognosis; the characteristics of the patients in the training set and the validation set were similar. The researchers found that five or more cells per 7.5 ml of blood were associated with poor prognosis. The data from the remaining 75 patients were used to validate that finding. At the initial sampling, 50% of the women with metastatic breast cancer had more than five CTCs per sample, with an average progression-free survival of less than three months and overall survival of 10 months. Women who had less than five CTCs per sample had a progression-free survival of seven months and an overall survival of 18 months. Treatment was initiated in the remaining patients, and samples were taken three to four weeks later. Only 30% of the women with metastatic breast cancer had five or more cells per sample, with an average progression-free survival of 2.1 months and overall survival of 8.2 months, compared to women who had less than five CTCs per sample, who had a progression-free survival of seven months and an overall survival of greater than 18 months. The number of cells per sample at follow-up was indicative of prognosis. Multivariate analysis of clinical factors demonstrated that while clinical factors (including time to metastasis, HER2/neu status and type of therapy) still correlated to outcomes; the strongest predictors of progression-free and overall survival were the levels of CTCs at baseline and at the first follow-up visit. This study suggests that levels of CTCs may be predictive of outcome in patients with malignant breast cancer.

Early experience with this technology was documented when researchers were able to distinguish carcinoma cells from other blood cells, and recovery of the breast carcinoma cells was 75–100% by (Racila, et al., 1998). Peripheral blood samples from 13 controls were compared to samples from 30 patients with breast cancer. In control specimens, the number of epithelial cells ranged from 0–5. Patients with localized breast cancer had an average of 15.9–17.4 epithelial cells per sample. In patients with spread to local lymph nodes only, the average number of cells was 47.4–52.3, and in patients with distant metastasis, the average number of cells was 122–140 per sample. The identification of cancer cells was confirmed by blinded evaluation of the cells by one of the researchers.

Literature Review for Circulating Tumor Cells in Prostate Cancer

Folkersma et al. (2012) reported on a prospective study that analyzed the correlation between circulating tumor cell (CTC) levels and clinicopathologic parameters (prostate-specific antigen [PSA] level, Gleason score, and TNM stage) in patients with metastatic hormone-sensitive prostate cancer (PCa) and to establish its prognostic value in overall survival (OS) and progression-free survival (PFS). The study included three arms: 30 patients with localized PCa; 30 patients with metastatic PCa; and, 30 healthy volunteers. The median follow-up was 42.9 months. A significant positive correlation was demonstrated between the CTC level and all tumor burden markers (PSA and T, N, and M stage; $P < .001$), except for Gleason score ($\text{tau} = 0.16$). A cutoff of ≥ 4 CTCs/7.5 mL was chosen to distinguish patients with a poor prognosis. These patients had a significantly shorter median OS and PFS (24 compared to 45 months and 7 compared to 44 months, respectively; $P < .001$). As the CTC level increased, the OS and PFS were noted to decrease. The risk of mortality and progression for the patients with ≥ 4 CTCs was 4.1 ($P = .029$) and 8.5 ($P < .001$) times greater. Multivariate analyses indicated that a CTC of ≥ 4 was an independent prognostic factor for PFS (hazard ratio 5.9, $P < .005$). The study is preliminary and needs to be confirmed in larger studies. Further studies need to be conducted to demonstrate the clinical utility of detection of CTC in prostate cancer.

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

Ali et al. (2010) reported on a study that assess the incidence of CTCs in 64 prostate cancer patients with low-volume tumors (less than 0.5 cc) after radical prostatectomy. Clinicopathological data and follow-up PSA data were compared to CTC status. Nine patients had 'low-volume prostate cancer' with seven of these patients with detectable levels of CTCs. PSA elevation was noted in two of the seven patients with detectable CTCs. CTCs may be associated with the presence of detectable PSA levels in the setting of low-volume prostate cancer.

Davis et al. (2008) reported on a study that evaluated the hypothesis that CTCs would correlate with tumor volume, pathological stage and Gleason score in men with localized prostate cancer. Blood was obtained from 97 men with localized prostate cancer before radical prostatectomy, and then on postoperative days two to three and at six weeks. Twenty-five men with an increased prostate specific antigen and no tumor detected on extended prostate biopsy served as a control group. Blood samples were analyzed for CTC with the CellSearch System. CTCs were detected in 21% of patients with cancer and 20% of controls ($p = 0.946$). At six weeks after prostatectomy, CTCs were detected in 16% and 11% ($p = 0.51$) of the men positive and negative for CTCs at baseline, respectively. Of the 20 patients with cancer who had CTCs at baseline, 18 did not have CTCs after surgery. CTC values did not correlate with tumor volume, pathological stage or Gleason score. Only 3.1% of the men with cancer and 8% of the control group had 3 or more CTCs per 22.5 ml blood at baseline. In metastatic breast, prostate and other cancers it appears that CTCs may correlate with prognosis. The authors note that in the setting of localized prostate cancer the number of detectable circulating tumor cells was low, with findings comparable to those in men who were biopsy negative for cancer. They note that the findings indicate no correlation between the number of circulating tumor cells and known prognostic factors in this population.

deBono et al. (2008) conducted a prospective study to evaluate the relationship between post-treatment CTC count and overall survival in castration-resistant prostate cancer (CRPC). Objectives also included determining the prognostic utility of CTC measurement before initiating therapy, and relationship of CTC to prostate-specific antigen (PSA) changes and overall survival at different end-points. The study involved 231 men with progressive disease. A blood sample was obtained when they were starting a new line of chemotherapy before treatment and monthly thereafter. Patients were divided into predetermined one of two groups: favorable or unfavorable (< 5 and ≥ 5 CTC/7.5ml). Patients in unfavorable pretreatment CTC (57%) had shorter overall survival (median

overall survival, 11.5 versus 21.7 months; Cox hazard ratio, 3.3; $p < 0.0001$). Unfavorable post-treatment CTC counts also predicted shorter overall survival at two to five, six to eight, nine to twelve, and 13 to 20 weeks (median overall survival, 6.7-9.5 versus 19.6-20.7 months; Cox hazard ratio, 3.6-6.5; $p < 0.0001$). overall survival was predicted with CTC counts better than with PSA decrement algorithms at all time points; area under the receiver operator curve for CTC was 81% to 87% and 58% to 68% for 30% PSA reduction ($p = 0.0218$). The prognosis for patients with (a) unfavorable baseline CTC who converted to favorable CTC improved (6.8 to 1.3 months); (b) favorable baseline CTC who converted to unfavorable worsened (>26 to 9.3 months).

Literature Review for Circulating Tumor Cells in Colon Cancer

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

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

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

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

Cohen et al. (2008; 2009) reported on prospective study that examined the role of CTC in predicting clinical outcomes in patients with metastatic colorectal cancer (mCRC). CTCs were counted in 430 patients with mCRC. Patients with unfavorable compared with favorable baseline CTCs had shorter median progression-free survival and overall survival. The differences persisted up to 20 weeks after therapy. The baseline and follow-up CTC levels remained strong predictors of progression-free survival and overall survival after adjustment for clinically significant factors. The study did not assess whether a change in therapy based on unfavorable CTCs is beneficial and further trials to explore this are warranted.

Sastre et al. (2008) examined the correlation of the presence of circulating tumor cells (CTCs) with the commonest clinical and morphological variables in patients with colon cancer. The study included 97 patients and 30 healthy volunteers. Blood was obtained and quantification of CTCs was performed with CellSearch System. Results of the testing were expressed in number of CTCs/7.5 ml and the cut-off of ≥ 2 CTCs was used to define the test as positive. Positive CTCs were detected in 34 of 94 patients (36.2%). A correlation was not found among positive CTCs and location of primary tumor, increased carcinoembryonic antigen level, increased lactate dehydrogenase level of grade of differentiation. Stage correlated with positive CTCs (20.7% in stage II; 24.1% in stage III; 60.7% in stage IV; $p=0.005$). Detection of CTCs correlated with stage but not with the other clinical and morphological variables.

Professional Societies/Organizations

American Society of Clinical Oncology (ASCO) 2007 Update of Recommendations for the Use of Tumor Markers in Breast Cancer: In their 2007 update, ASCO made the following recommendations regarding testing for circulating tumor cells (CTCs) in breast cancer (Harris, et al., 2007):

- The measurement of CTCs should not be used to make the diagnosis of breast cancer or to influence any treatment decisions in patients with breast cancer.
- The use of CellSearch Assay in patients with metastatic breast cancer cannot be recommended until additional validation confirms the clinical value of this test.
- There are no data yet generated to prove that the use of CTCs testing will lead to a longer survival time or improved quality of life for the patient with metastatic breast cancer.
- While studies of CTCs testing of patients with early-stage breast cancer suggest their potential utility, additional studies are necessary to determine the utility of CTCs in early breast cancer.

The National Academy of Clinical Biochemistry (NACB): the NACB published Laboratory Medicine Practice Guidelines for Use of Tumor Markers in Testicular, Prostate, Colorectal, Breast, and Ovarian Cancers (NACB, 2009). The guideline includes the following recommendations:

- Regarding measurement of circulating prostate cancer cells in peripheral blood, it is noted, "While initial results are encouraging, these techniques are not yet sufficiently validated to warrant recommendations their application in routine clinical practice."
- Regarding detection of tumor cells in circulation in breast cancer, it is noted that, this testing is undergoing evaluation; it is available but not widely used in clinical practice; and prospective randomized trial are underway.

Use Outside of the US

The European Society for Medical Oncology (ESMO) published clinical practice guidelines for diagnosis, treatment and follow-up of locally recurrent or metastatic breast cancer (Cardosa, et al., 2012). Regarding circulating tumor cell testing, the guidelines note, "Circulating tumor cells are still an experimental technique and should not be used outside a clinical trial."

Summary

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

than any existing tumor markers in its efficacy and clinical utility. The role of this testing in patient management is not yet known.

Coding/Billing Information

Note: 1) This list of codes may not be all-inclusive.

2) Deleted codes and codes which are not effective at the time the service is rendered may not be eligible for reimbursement

Experimental/Investigational/Unproven/Not Covered:

| CPT* Codes | Description |
|------------|--|
| 86152 | Cell enumeration using immunologic selection and identification in fluid specimen (eg, circulating tumor cells in blood) |
| 86153 | Cell enumeration using immunologic selection and identification in fluid specimen (eg, circulating tumor cells in blood); physician interpretation and report, when required |

*Current Procedural Terminology (CPT®) ©2013 American Medical Association: Chicago, IL.

References

1. Aguado J. Identification of circulating tumour cells in metastatic breast cancer with the CellSearch™ system. Technological Report. Seville, Spain: Agencia de Evaluacion de Tecnologias Sanitarias de Andalucia (AETSA); 2006.
2. Alemar J, Schuur ER. Progress in using circulating tumor cell information to improve metastatic breast cancer therapy. *J Oncol.* 2013;2013:702732. doi:10.1155/2013/702732. Epub 2013 Mar 25.
3. Ali A, Furusato B, Ts'o PO, Lum ZP, Elsamanoudi S, Mohamed A, Srivastava S, et al. Assessment of circulating tumor cells (CTCs) in prostate cancer patients with low-volume tumors. *Pathol Int.* 2010 Oct;60(10):667-72.
4. Allan AL, Keeney M. Circulating tumor cell analysis: technical and statistical considerations for application to the clinic. *J Oncol.* 2010;2010:426218. Epub 2009 Dec 13.
5. Allard WJ, Matera J, Miller MC, Repollet M, Connelly MC, Rao C, et al. Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. *Clin Cancer Res.* 2004 Oct 15;10(20):6897-904.
6. American Cancer Society (ACS). Tumor markers. Last Medical Review: 10/30/2013. Accessed January 2, 2014. Available at URL address: http://www.cancer.org/docroot/PED/content/PED_2_3X_Tumor_Markers.asp?sitearea=PED
7. Balic M, Dandachi N, Hofmann G, Samonigg H, Loibner H, Obwaller A, et al. Comparison of two methods for enumerating circulating tumor cells in carcinoma patients. *Cytometry B Clin Cytom.* 2005 Nov;68(1):25-30.
8. Bidard FC, Mathiot C, Delalogue S, Brain E, Giachetti S, de Cremoux P, et al. Single circulating tumor cell detection and overall survival in nonmetastatic breast cancer. *Ann Oncol.* 2010 Apr;21(4):729-33.(a)

9. Bidard FC, Mathiot C, Degeorges A, Etienne-Grimaldi MC, Delva R, Pivot X, et al. Clinical value of circulating endothelial cells and circulating tumor cells in metastatic breast cancer patients treated first line with bevacizumab and chemotherapy. *Ann Oncol*. 2010 Sep;21(9):1765-71. (b)
10. Bidard FC, Fehm T, Ignatiadis M, Smerage JB, Alix-Panabières C, Janni W, et al. Clinical application of circulating tumor cells in breast cancer: overview of the current interventional trials. *Cancer Metastasis Rev*. 2012 Nov 6. [Epub ahead of print] (a)
11. Bidard FC, Hajage D, Bachelot T, Delaloge S, Brain E, Campone M, et al. Assessment of circulating tumor cells and serum markers for progression-free survival prediction in metastatic breast cancer: a prospective observational study. *Breast Cancer Res*. 2012 Feb 13;14(1):R29. (b)
12. Botteri E, Sandri MT, Bagnardi V, Munzone E, Zorzino L, Rotmensz N, et al. Modeling the relationship between circulating tumour cells number and prognosis of metastatic breast cancer. *Breast Cancer Res Treat*. 2010 Jul;122(1):211-7.
13. Budd GT, Cristofanilli M, Ellis MJ, Stopeck A, Borden E, Miller MC, et al. Circulating tumor cells versus imaging--predicting overall survival in metastatic breast cancer. *Clin Cancer Res*. 2006 Nov 1;12(21):6403-9.
14. Cardoso F, Harbeck N, Fallowfield L, Kyriakides S, Senkus E; ESMO Guidelines Working Group. Locally recurrent or metastatic breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2012 Oct;23 Suppl 7:vii11-9.
15. Chan F, Goodman O, Fink L, Vogelzang NJ, Pomerantz D, Khoury JD. Dramatically elevated circulating tumor cell numbers in a patient with small cell neuroendocrine carcinoma of the prostate. *Arch Pathol Lab Med*. 2010 Jan;134(1):120-3.
16. Cohen SJ, Punt CJ, Iannotti N, Saidman BH, Sabbath KD, Gabrail NY, et al. Prognostic significance of circulating tumor cells in patients with metastatic colorectal cancer. *Ann Oncol*. 2009 Jul;20(7):1223-9.
17. Cohen SJ, Punt CJ, Iannotti N, Saidman BH, Sabbath KD, Gabrail NY, et al. Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. *J Clin Oncol*. 2008 Jul 1;26(19):3213-21.
18. College of American Pathologists (CAP). Developed by CAP's Technology Assessment Committee. POET REPORT. Perspectives on Emerging Technology. Circulating Tumor Cells (CTCs). November, 2010.
19. Criscitiello C, Sotiriou C, Ignatiadis M. Circulating tumor cells and emerging blood biomarkers in breast cancer. *Curr Opin Oncol*. 2010 Nov;22(6):552-8.
20. Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Matera J, Miller MC, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. *N Engl J Med*. 2004 Aug 19;351:781-91.
21. Cristofanilli M, Hayes DF, Budd GT, Ellis MJ, Stopeck A, Reuben JM, Circulating tumor cells: a novel prognostic factor for newly diagnosed metastatic breast cancer. *J Clin Oncol*. 2005 Mar 1;23(7):1420-30. Erratum in: *J Clin Oncol*. 2005 Jul 20;23(21):4808.
22. Cristofanilli M, Broglio KR, Guarneri V, Jackson S, Fritsche HA, Islam R, et al. Circulating tumor cells in metastatic breast cancer: biologic staging beyond tumor burden. *Clin Breast Cancer*. 2007 Feb;7(6):471-9.
23. Danila DC, Heller G, Gignac GA, Gonzalez-Espinoza R, Anand A, Tanaka E, et al. Circulating tumor cell number and prognosis in progressive castration-resistant prostate cancer. *Clin Cancer Res*. 2007 Dec 1;13(23):7053-8.

24. Davis JW, Nakanishi H, Kumar VS, Bhadkamkar VA, McCormack R, Fritsche HA, et al. Circulating tumor cells in peripheral blood samples from patients with increased serum prostate specific antigen: initial results in early prostate cancer. *J Urol*. 2008 Jun;179(6):2187-91; discussion 2191.
25. Dawood S, Broglio K, Valero V, Reuben J, Handy B, Islam R, et al. Circulating tumor cells in metastatic breast cancer: from prognostic stratification to modification of the staging system? *Cancer*. 2008 Nov 1;113(9):2422-30.
26. de Bono JS, Scher HI, Montgomery RB, Parker C, Miller MC, Tissing H, et al. Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. *Clin Cancer Res*. 2008 Oct 1;14(19):6302-9.
27. De Giorgi U, Valero V, Rohren E, Dawood S, Ueno NT, Miller MC, et al. Circulating tumor cells and [18F]fluorodeoxyglucose positron emission tomography/computed tomography for outcome prediction in metastatic breast cancer. *J Clin Oncol*. 2009 Jul 10;27(20):3303-11.
28. De Giorgi U, Valero V, Rohren E, Mego M, Doyle GV, Miller MC, et al. Circulating tumor cells and bone metastases as detected by FDG-PET/CT in patients with metastatic breast cancer. *Ann Oncol*. 2010 Jan;21(1):33-9.
29. Fehm T, Hoffmann O, Aktas B, Becker S, Solomayer EF, Wallwiener D, et al. Detection and characterization of circulating tumor cells in blood of primary breast cancer patients by RT-PCR and comparison to status of bone marrow disseminated cells. *Breast Cancer Res*. 2009;11(4):R59.
30. Gallagher DJ, Milowsky MI, Ishill N, Trout A, Boyle MG, Riches J, et al. Detection of circulating tumor cells in patients with urothelial cancer. *Ann Oncol*. 2008 Oct 3.
31. Gazzaniga P, Naso G, Gradilone A, Cortesi E, Gandini O, Gianni W, et al. Chemosensitivity profile assay of circulating cancer cells: prognostic and predictive value in epithelial tumors. *Int J Cancer*. 2010 May 15;126(10):2437-47.
32. Gerges N, Rak J, Jabado N. New technologies for the detection of circulating tumour cells. *Br Med Bull*. 2010;94:49-64. Epub 2010 Apr 23.
33. Giordano A, Giuliano M, De Laurentiis M, Arpino G, Jackson S, Handy BC, et al. Circulating tumor cells in immunohistochemical subtypes of metastatic breast cancer: lack of prediction in HER2-positive disease treated with targeted therapy. *Ann Oncol*. 2012 May;23(5):1144-50.
34. Goodman OB Jr, Fink LM, Symanowski JT, Wong B, Grobaski B, Pomerantz D, et al. Circulating tumor cells in patients with castration-resistant prostate cancer baseline values and correlation with prognostic factors. *Cancer Epidemiol Biomarkers Prev*. 2009 Jun;18(6):1904-13.
35. Gradilone A, Naso G, Raimondi C, Cortesi E, Gandini O, Vincenzi B, et al. Circulating tumor cells (CTCs) in metastatic breast cancer (MBC): prognosis, drug resistance and phenotypic characterization. *Ann Oncol*. 2011 Jan;22(1):86-92.
36. Giuliano M, Giordano A, Jackson S, Hess KR, De Giorgi U, Mego M, et al. Circulating tumor cells as prognostic and predictive markers in metastatic breast cancer patients receiving first-line systemic treatment. *Breast Cancer Res*. 2011 Jun 15;13(3):R67.
37. Groot Koerkamp B, Rahbari NN, Büchler MW, Koch M, Weitz J. Circulating tumor cells and prognosis of patients with resectable colorectal liver metastases or widespread metastatic colorectal cancer: a meta-analysis. *Ann Surg Oncol*. 2013 Jul;20(7):2156-65.

38. Harris L, Fritsche H, Mennel R, Norton L, Ravdin P, Taube S, et al. ; American Society of Clinical Oncology. American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. *J Clin Oncol*. 2007 Nov 20;25(33):5287-312.
39. Hayes DF, Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Miller MC, et al. Circulating tumor cells at each follow-up time point during therapy of metastatic breast cancer patients predict progression-free and overall survival. *Clin Cancer Res*. 2006 Jul 15;12(14 Pt 1):4218-24.
40. Hiltermann TJ, Pore MM, van den Berg A, Timens W, Boezen HM, Liesker JJ, et al. Circulating tumor cells in small-cell lung cancer: a predictive and prognostic factor. *Ann Oncol*. 2012 Nov;23(11):2937-42.
41. Hiraiwa K, Takeuchi H, Hasegawa H, Saikawa Y, Suda K, Ando T, et al. Clinical significance of circulating tumor cells in blood from patients with gastrointestinal cancers. *Ann Surg Oncol*. 2008 Nov;15(11):3092-100.
42. Ignatiadis M, Georgoulis V, Mavroudis D. Circulating tumor cells in breast cancer. *Curr Opin Obstet Gynecol*. 2008 Feb;20(1):55-60.
43. Jacob J, Krell J, Castellano L, Jiao LR, Stebbing J, Frampton AE. Determination of cut-offs for circulating tumor cell measurement in metastatic cancer. *Expert Rev Anticancer Ther*. 2011 Sep;11(9):1345-50.
44. Jost M, Day JR, Slaughter R, Koreckij TD, Gonzales D, Kinnunen M, et al. Molecular assays for the detection of prostate tumor derived nucleic acids in peripheral blood. *Mol Cancer*. 2010 Jul 2;9:174.
45. Kahn HJ, Presta A, Yang LY, Blondal J, Trudeau M, Lickley L, et al. Enumeration of circulating tumor cells in the blood of breast cancer patients after filtration enrichment: correlation with disease stage. *Breast Cancer Res Treat*. 2004 Aug;86(3):237-47.
46. Krebs MG, Hou JM, Ward TH, Blackhall FH, Dive C. Circulating tumour cells: their utility in cancer management and predicting outcomes. *Ther Adv Med Oncol*. 2010 Nov;2(6):351-65.
47. Lianidou ES, Markou A, Strati A. Molecular characterization of circulating tumor cells in breast cancer: challenges and promises for individualized cancer treatment. *Cancer Metastasis Rev*. 2012 Dec;31(3-4):663-71.
48. Liu MC, Shields PG, Warren RD, Cohen P, Wilkinson M, Ottaviano YL, et al. Circulating tumor cells: a useful predictor of treatment efficacy in metastatic breast cancer. *J Clin Oncol*. 2009 Nov 1;27(31):5153-9.
49. Lucci A, Hall CS, Lodhi AK, Bhattacharyya A, Anderson AE, Xiao L, et al. Circulating tumour cells in non-metastatic breast cancer: a prospective study. *Lancet Oncol*. 2012 Jul;13(7):688-95.
50. Ma XL, Xiao ZL, Liu L, Liu XX, Nie W, Li P, Chen NY, Wei YQ. Meta-analysis of circulating tumor cells as a prognostic marker in lung cancer. *Asian Pac J Cancer Prev*. 2012;13(4):1137-44.
51. Maheswaran S, Haber DA. Circulating tumor cells: a window into cancer biology and metastasis. *Curr Opin Genet Dev*. 2010 Feb;20(1):96-9.
52. Mego M, De Giorgi U, Broglio K, Dawood S, Valero V, Andreopoulou E, et al. Circulating tumour cells are associated with increased risk of venous thromboembolism in metastatic breast cancer patients. *Br J Cancer*. 2009 Dec 1;101(11):1813-6.
53. Mego M, De Giorgi U, Hsu L, Ueno NT, Valero V, Jackson S, et al. Circulating tumor cells in metastatic inflammatory breast cancer. *Ann Oncol*. 2009 Nov;20(11):1824-8.

54. Moreno JG, Miller MC, Gross S, Allard WJ, Gomella LG, Terstappen LW. Circulating tumor cells predict survival in patients with metastatic prostate cancer. *Urology*. 2005 Apr;65(4):713-8.
55. Müller V, Riethdorf S, Rack B, Janni W, Fasching PA, Solomayer E, et al.; on behalf of the DETECT study group. Prognostic impact of circulating tumor cells assessed with the CellSearch System™ and AdnaTest Breast™ in metastatic breast cancer patients: the DETECT study. *Breast Cancer Res*. 2012 Aug 15;14(4):R118. [Epub ahead of print]
56. Nadal R, Fernandez A, Sanchez-Rovira P, Salido M, Rodríguez M, García-Puche JL, et al. Biomarkers characterization of circulating tumour cells in breast cancer patients. *Breast Cancer Res*. 2012 May 3;14(3):R71.
57. Nagaiah G, Abraham J. Circulating tumor cells in the management of breast cancer. *Clin Breast Cancer*. 2010 Jun;10(3):209-16.
58. Nakamura S, Yagata H, Ohno S, Yamaguchi H, Iwata H, Tsunoda N, et al. Multi-center study evaluating circulating tumor cells as a surrogate for response to treatment and overall survival in metastatic breast cancer. *Breast Cancer*. 2010 Jul;17(3):199-204.
59. National Academy of Clinical Biochemistry (NACB). Laboratory Medicine Practice Guidelines for use of tumor markers in testicular, prostate, colorectal, breast, and ovarian cancers. Sturgeon CM, Diamandis EP editors. 2009. Accessed January 2, 2014. Available at URL address: <http://www.aacc.org/members/nacb/LMPG/OnlineGuide/PublishedGuidelines/major/Pages/toc.aspx>
60. National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology. NCCN Clinical Practice Guidelines in Oncology™. Breast Cancer. V.1.2014. Accessed January 5, 2014. Available at URL address: http://www.nccn.org/professionals/physician_gls/f_guidelines.asp
61. National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology. NCCN Clinical Practice Guidelines in Oncology™. Colon Cancer. V.2.2014. Accessed January 5, 2014. Available at URL address: http://www.nccn.org/professionals/physician_gls/f_guidelines.asp
62. National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology. NCCN Clinical Practice Guidelines in Oncology™. Prostate Cancer. V.1.2014. Accessed January 5, 2014. Available at URL address: http://www.nccn.org/professionals/physician_gls/f_guidelines.asp
63. National Comprehensive Cancer Network®, Inc., (NCCN®), All Rights Reserved. NCCN Task Force® report: Evaluating the clinical utility of tumor markers in oncology panel members. Vol 9 Supp 5. Nov 2011. Accessed January 5, 2014. Available at URL address: <http://www.nccn.org/JNCCN/supplements.asp>
64. Nolé F, Munzone E, Zorzino L, Minchella I, Salvatici M, Botteri E, et al. Variation of circulating tumor cell levels during treatment of metastatic breast cancer: prognostic and therapeutic implications. *Ann Oncol*. 2007 Dec 4.
65. Okegawa T, Nutahara K, Higashihara E. Prognostic significance of circulating tumor cells in patients with hormone refractory prostate cancer. *J Urol*. 2009 Mar;181(3):1091-7.
66. Okegawa T, Nutahara K, Higashihara E. Immunomagnetic quantification of circulating tumor cells as a prognostic factor of androgen deprivation responsiveness in patients with hormone naive metastatic prostate cancer. *J Urol*. 2008 Oct;180(4):1342-7.
67. Olmos D, Arkenau HT, Ang JE, Ledaki I, Attard G, Carden CP, et al. Circulating tumour cell (CTC) counts as intermediate end points in castration-resistant prostate cancer (CRPC): a single-centre experience. *Ann Oncol*. 2009 Jan;20(1):27-33.

68. OncoQuick™ product information. Greiner Bio-One, Inc. Accessed January 5, 2014. Available at URL address: http://www.greinerbioone.com/en/row/articles/catalogue/article-groups/283_11/
69. Pantel K, Alix-Panabières C, Riethdorf S. Cancer micrometastases. *Nat Rev Clin Oncol*. 2009 Jun;6(6):339-51.
70. Panteleakou Z, Lembessis P, Sourla A, Pissimissis N, Polyzos A, Deliveliotis C, Koutsilieris M. Detection of circulating tumor cells in prostate cancer patients: methodological pitfalls and clinical relevance. *Mol Med*. 2008 Dec 12.
71. Park Y, Kitahara T, Urita T, Yoshida Y, Kato R. Expected clinical applications of circulating tumor cells in breast cancer. *World J Clin Oncol*. 2011 Aug;2(8):303-10.
72. Patel AS, Allen JE, Dicker DT, Peters KL, Sheehan JM, Glantz MJ, El-Deiry WS. Identification and enumeration of circulating tumor cells in the cerebrospinal fluid of breast cancer patients with central nervous system metastases. *Oncotarget*. 2011 Oct;2(10):752-60.
73. Petrelli NJ, Winer EP, Brahmer J, Dubey S, Smith S, Thomas C, et al. Clinical Cancer Advances 2009: major research advances in cancer treatment, prevention, and screening--a report from the American Society of Clinical Oncology. *J Clin Oncol*. 2009 Dec 10;27(35):6052-69.
74. Pierga JY, Hajage D, Bachelot T, Delaloge S, Brain E, Campone M, et al. High independent prognostic and predictive value of circulating tumor cells compared with serum tumor markers in a large prospective trial in first-line chemotherapy for metastatic breast cancer patients. *Ann Oncol*. 2012 Mar;23(3):618-24. doi: 10.1093/annonc/mdr263.
75. Racila E, Euhus D, Weiss AJ, Rao C, McConnell J, Terstappen LW, Uhr JW. Detection and characterization of carcinoma cells in the blood. *Proc Natl Acad Sci U S A*. 1998 Apr;95:4589-94.
76. Rahbari NN, Aigner M, Thorlund K, Mollberg N, Motschall E, Jensen K, et al. Meta-analysis shows that detection of circulating tumor cells indicates poor prognosis in patients with colorectal cancer. *Gastroenterology*. 2010 May;138(5):1714-26.
77. Resel Folkersma L, San José Manso L, Galante Romo I, Moreno Sierra J, Olivier Gómez C. Prognostic significance of circulating tumor cell count in patients with metastatic hormone-sensitive prostate cancer. *Urology*. 2012 Dec;80(6):1328-32.
78. Riethdorf S, Fritsche H, Müller V, Rau T, Schindlbeck C, Rack B, et al. Detection of circulating tumor cells in peripheral blood of patients with metastatic breast cancer: a validation study of the CellSearch system. *Clin Cancer Res*. 2007 Feb 1;13(3):920-8.
79. Riethdorf S, Pantel K. Advancing personalized cancer therapy by detection and characterization of circulating carcinoma cells. *Ann N Y Acad Sci*. 2010 Oct;1210:66-77.
80. Ring A, Smith IE, Dowsett M. Circulating tumour cells in breast cancer. *Lancet Oncol*. 2004 Feb;5(2):79-88.
81. Ring AE, Zabaglo L, Ormerod MG, Smith IE, Dowsett M. Detection of circulating epithelial cells in the blood of patients with breast cancer: comparison of three techniques. *Br J Cancer*. 2005 Mar 14;92(5):906-12.
82. Ross JS, Slodkowska EA. Circulating and disseminated tumor cells in the management of breast cancer. *Am J Clin Pathol*. 2009 Aug;132(2):237-45.
83. Saad A, Kanate A, Sehbai A, Marano G, Hobbs G, Abraham J. Correlation among [18F]fluorodeoxyglucose positron emission tomography/computed tomography, cancer antigen 27.29, and circulating tumor cell testing in metastatic breast cancer. *Clin Breast Cancer*. 2008 Aug;8(4):357-61.

84. Sastre J, Maestro ML, Puente J, Veganzones S, Alfonso R, Rafael S, et al. Circulating tumor cells in colorectal cancer: correlation with clinical and pathological variables. *Ann Oncol*. 2008 May;19(5):935-8.
85. Sastre J, Maestro ML, Gómez-España A, Rivera F, Valladares M, Massuti B, et al. Circulating tumor cell count is a prognostic factor in metastatic colorectal cancer patients receiving first-line chemotherapy plus bevacizumab: a Spanish Cooperative Group for the Treatment of Digestive Tumors study. *Oncologist*. 2012;17(7):947-55.
86. Scher HI, Jia X, de Bono JS, Fleisher M, Pienta KJ, Raghavan D, Heller G. Circulating tumour cells as prognostic markers in progressive, castration-resistant prostate cancer: a reanalysis of IMMC38 trial data. *Lancet Oncol*. 2009 Mar;10(3):233-9.
87. Scher HI, Morris MJ, Basch E, Heller G. End points and outcomes in castration-resistant prostate cancer: from clinical trials to clinical practice. *J Clin Oncol*. 2011 Sep 20;29(27):3695-704.
88. Serrano MJ, Rovira PS, Martínez-Zubiaurre I, Rodriguez MD, Fernández M, Lorente JA. Dynamics of circulating tumor cells in early breast cancer under neoadjuvant therapy. *Exp Ther Med*. 2012 Jul;4(1):43-48. Epub 2012 Apr 5.
89. Sieuwerts AM, Kraan J, Bolt J, van der Spoel P, Elstrodt F, Schutte M, et al. Anti-epithelial cell adhesion molecule antibodies and the detection of circulating normal-like breast tumor cells. *J Natl Cancer Inst*. 2009 Jan 7;101(1):61-6.
90. Shaffer DR, Leversha MA, Danila DC, Lin O, Gonzalez-Espinoza R, Gu B, et al. Circulating tumor cell analysis in patients with progressive castration-resistant prostate cancer. *Clin Cancer Res*. 2007 Apr 1;13(7):2023-9.
91. Slade MJ, Payne R, Riethdorf S, Ward B, Zaidi SA, Stebbing J, et al. Comparison of bone marrow, disseminated tumour cells and blood-circulating tumour cells in breast cancer patients after primary treatment. *Br J Cancer*. 2009 Jan 13;100(1):160-6.
92. Smerage JB, Hayes DF. The measurement and therapeutic implications of circulating tumour cells in breast cancer. *Br J Cancer*. 2006 Jan 16;94(1):8-12.
93. Terstappen LW, Rao C, Gross S, Weiss AJ. Peripheral blood tumor cell load reflects the clinical activity of the disease in patients with carcinoma of the breast. *Int J Oncol*. 2000 Sep;17(3):573-8.
94. Tewes M, Aktas B, Welt A, Mueller S, Hauch S, Kimmig R, et al. Molecular profiling and predictive value of circulating tumor cells in patients with metastatic breast cancer: an option for monitoring response to breast cancer related therapies. *Breast Cancer Res Treat*. 2009 Jun;115(3):581-90.
95. Thorsteinsson M, Jess P. The clinical significance of circulating tumor cells in non-metastatic colorectal cancer--a review. *Eur J Surg Oncol*. 2011 Jun;37(6):459-65. Epub 2011 Feb 15.
96. Tibbe AG, Miller MC, Terstappen LW. Statistical considerations for enumeration of circulating tumor cells. *Cytometry A*. 2007 Mar;71(3):154-62.
97. Tokudome N, Ito Y, Takahashi S, Kobayashi K, Taira S, Tsutsumi C, et al. Detection of circulating tumor cells in peripheral blood of heavily treated metastatic breast cancer patients. *Breast Cancer*. 2011 Jul;18(3):195-202. Epub 2011 Apr 5.
98. Tol J, Koopman M, Miller MC, Tibbe A, Cats A, Creemers GJ, et al. Circulating tumour cells early predict progression-free and overall survival in advanced colorectal cancer patients treated with chemotherapy and targeted agents. *Ann Oncol*. 2010 May;21(5):1006-12.

99. U.S. Food and Drug Administration (FDA). New device clearance. CellSearch™ epithelial cell kit/CellSpotter analyzer – K031588. Accessed January 5, 2014. Available at URL address: <http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/DeviceApprovalsandClearances/Recently-ApprovedDevices/ucm081239.htm>
100. U.S. Food and Drug Administration (FDA). Federal Register: May 11, 2004 (Volume 69, Number 91. Medical Devices; Immunology and Microbiology Devices; Classification of the Immunomagnetic Circulating Cancer Cell Selection and Enumeration System. Accessed January 5, 2014. Available at URL address: <http://www.fda.gov/OHRMS/DOCKETS/98fr/04-10592.htm>
101. U.S. Food and Drug Administration (FDA). Class II Special Controls Guidance Document: Immunomagnetic Circulating Cancer Cell Selection and Enumeration System. May 11, 2004. Accessed January 5, 2014. Available at URL address: <http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm077688.htm>
102. U.S. Food and Drug Administration (FDA). CellSearch Circulating Tumor Cell Kit. Premarket Notification- Expanded Indications for Use-Metastatic Prostate Cancer. K073338. Feb. 27, 2008.
103. U.S. Food and Drug Administration (FDA). CellSearch Circulating Tumor Cell Kit. Premarket Notification- Expanded Indications for Use-Metastatic Colon Cancer. K071729. Nov. 26, 2007.
104. Vishnu P, Tan WW. Update on options for treatment of metastatic castration-resistant prostate cancer. *Onco Targets Ther.* 2010 Jun 24;3:39-51.
105. Wallwiener M, Hartkopf AD, Baccelli I, Riethdorf S, Schott S, Pantel K, et al. The prognostic impact of circulating tumor cells in subtypes of metastatic breast cancer. *Breast Cancer Res Treat.* 2013 Jan;137(2):503-10. doi: 10.1007/s10549-012-2382-0. Epub 2012 Dec 28.
106. Wang FB, Yang XQ, Yang S, Wang BC, Feng MH, Tu JC. A higher number of circulating tumor cells (CTC) in peripheral blood indicates poor prognosis in prostate cancer patients--a meta-analysis. *Asian Pac J Cancer Prev.* 2011;12(10):2629-35.
107. Weigel MT, Dowsett M. Current and emerging biomarkers in breast cancer: prognosis and prediction. *Endocr Relat Cancer.* 2010 Sep 23;17(4):R245-62.
108. Wong NS, Kahn HJ, Zhang L, Oldfield S, Yang LY, Marks A, et al. Prognostic significance of circulating tumour cells enumerated after filtration enrichment in early and metastatic breast cancer patients. *Breast Cancer Res Treat.* 2006 Sep;99(1):63-9. Epub 2006 Mar 16.
109. Yagata H, Nakamura S, Toi M, Bando H, Ohno S, Kataoka A. Evaluation of circulating tumor cells in patients with breast cancer: multi-institutional clinical trial in Japan. *Int J Clin Oncol.* 2008 Jun;13(3):252-6.
110. Zhang L, Riethdorf S, Wu G, Wang T, Yang K, Peng G, Liu J, Pantel K. Meta-analysis of the prognostic value of circulating tumor cells in breast cancer. *Clin Cancer Res.* 2012 Oct 15;18(20):5701-10.

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