**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION**
**DECISION SUMMARY**
**ASSAY ONLY TEMPLATE**

## A. 510(k) Number:

K052191

## B. Purpose for Submission:

The following labeling changes were made: 1) change in concentration of one kit reagent; 2) change in storage condition of another reagent; 3) addition of further data analysis for the clinical study; and 4) addition of related limitations statement. No change was made to the intended use of the test.

## C. Measurand:

EpCam, Cytokeratins 8, 18 and/or 19, and CD45 to identify Circulating Tumor Cells (CTC) (Cells appearing to look like tumor cells with epithelial cell markers and no lymphocyte marker on their surfaces).

## D. Type of Test:

A semi-automated qualitative immunomagnetic-capture immunofluorescent detection image analysis test.

## E. Applicant:

Veridex, LLC, A Johnson and Johnson Company

## F. Proprietary and Established Names:

CellSearch™ Circulating Tumor Cell Kit (Epithelial)

## G. Regulatory Information:

1. Regulation section:

   21 CFR 866.6020-Immunomagnetic Circulating Cancer Cell Selection and Enumeration System Immunomagnetic circulating cancer cell selection and enumeration systems are devices consisting of biological probes, fluorochromes, and other reagents; preservation and preparation devices; and a semi-automated analytical instrument to select and count circulating cancer cells in a prepared sample of whole blood. This device is intended for adjunctive use in monitoring or predicting cancer disease progression, response to therapy, and for the

detection of recurrent disease.

2. Classification:

   Class II

3. Product code:

   NQI

4. Panel:

   Immunology 82

## H. Intended Use:

1. Intended use(s):

   The CellSearch™ Circulating Tumor Cell Kit is intended for the enumeration of circulating tumor cells (CTC) of epithelial origin (CD45-, EpCAM+, and cytokeratins 8, 18+, and/or 19+) in whole blood.

2. Indication(s) for use:

   The presence of CTC in the peripheral blood, as detected by the CellSearch™ Circulating Tumor Cell Kit, is associated with decreased progression free survival and decreased overall survival in patients treated for metastatic breast cancer. A CTC count of 5 or more per 7.5 mL of blood is predictive of shorter progression free survival and overall survival.

3. Special conditions for use statement(s):

   For prescription use only.

4. Special instrument requirements:

   The CellTracks® AutoPrep system and the CellTracks® Analyzer II. The CellTracks® Analyzer II is a semi-automated fluorescence microscope intended to enumerate fluorescently labeled cells that are immunomagnetically selected and distributed over a viewing surface

## I. Device Description:

The CellSearch™ Circulating Tumor Cell Kit contains a ferrofluid-based capture reagent and immunofluorescent reagents. The ferrofluid reagent consists of nanoparticles with a magnetic core surrounded by a polymeric layer coated with

antibodies targeting the EpCAM antigen for capturing CTC. After immunomagnetic capture and enrichment, fluorescent reagents are added for identification and enumeration of CTC. The fluorescent reagents include the following: anti-CK-Phycoerythrin (PE) specific for the intracellular protein cytokeratin (characteristic of epithelial cells), DAPI which stains the cell nucleus, and anti-CD45-Allophycocyanin (APC) specific for leukocytes

The reagent/sample mixture is dispensed by the CellTracks® AutoPrep System into a cartridge that is inserted into a MagNest® cell presentation device. The strong magnetic field of the MagNest® device attracts the magnetically labeled epithelial cells to the surface of the cartridge. The CellTracks® Analyzer II or CellSpotter® Analyzer automatically scans the entire surface of the cartridge, acquires images and displays any event to the user where CK-PE and DAPI fluorescence are co-located. Images are presented to the user in a gallery format for final classification. An event is classified as a tumor cell when its morphological features are consistent with that of a tumor cell and it exhibits the phenotype EpCAM+, CK+, DAPI+ and CD45-

**J. Substantial Equivalence Information:**

1. Predicate device name(s):

   CellSearch™ Circulating Tumor Cell Kit (Epithelial)

2. Predicate 510(k) number(s):

   K050245

3. Comparison with predicate of modifications made to reagents:

| **Similarities** | | |
|---|---|---|
| Item | Predicate | Device e |
| | CellSearch™ Circulating Tumor Cell Kit (Epithelial) (K050245) | CellSearch™ Circulating Tumor Cell Kit (Epithelial) (K052191) |
| Manufacturer | Veridex, LLC<br>Warren, New Jersey | Same |
| Device Classification and Fundamental Scientific Technology | Immunomagnetic Circulating Cancer Cell Selection and Enumeration System | Same |
| Intended Use | The CellSearch™ Circulating Tumor Cell Kit is intended for the enumeration of circulating | Same |

| Similarities | | |
|---|---|---|
| Item | Predicate | Device e |
| | tumor cells (CTC) of epithelial origin (CD45-, EpCAM+, and cytokeratins 8, 18+, and/or 19+) in whole blood.<br><br>The presence of CTC in the peripheral blood, as detected by the CellSearch™ Circulating Tumor Cell Kit, is associated with decreased progression free survival and decreased overall survival in patients treated for metastatic breast cancer. A CTC count of 5 or more per 7.5mL of blood is predictive of shorter progression free survival and overall survival. | |
| Indications for Use | The CellSearch™ Circulating Tumor Cell Kit is intended for the enumeration of circulating tumor cells (CTC) of epithelial origin (CD45-, EpCAM+ and cytokeratin 8 & 18+, and/or cytokeratin 19+) in whole blood in conjunction with the CellTracks® AutoPrep System, the CellSpotter® Analyzer or CellTracks® Analyzer II, and the CellSearch™ Circulating Tumor Cell Control Kit.<br><br>The presence of CTC in the peripheral blood, as detected by the CellSearch™ Circulating Tumor Cell Kit, is associated with decreased progression free survival and decreased overall survival in patients treated | Same |

| Similarities | | |
|---|---|---|
| Item | Predicate | Device e |
| | for metastatic breast cancer. A CTC count of 5 or more per 7.5 mL of blood generally is predictive of shorter progression free survival and shorter overall survival. | |
| CellSearch™ Circulating Tumor Cell Kit Reagents | Anti-EpCAM Ferrofluid, Nucleic Acid Dye, Capture Enhancement Reagent, Permeabilization Reagent, Cell Fixative. | Same |

| Differences | | |
|---|---|---|
| Item | Predicate | Device |
| **Summary of labeling changes** | CellSearch™ Circulating Tumor Cell Kit (Epithelial) (K050245) | CellSearch™ Circulating Tumor Cell Kit (Epithelial) (K052191) |
| CHANGE #1 CELLSEARCH STAINING REAGENT Modification: Monoclonal antibody change from 0.0008% to 0.0006%. | **3.0 mL bottle Staining Reagent**:<br>Contains 0.0008% mouse monoclonal antibodies specific to cytokeratins conjugated to phycoerythrin (PE); 0.0012% mouse anti-CD45 mouse monoclonal antibody conjugated to allophycocyanin (APC) in phosphate buffered saline (PBS) containing 0.5% BSA, and 0.1% sodium azide. (white cap) | **3.0 mL bottle Staining Reagent**:<br>Contains 0.0006% mouse monoclonal antibodies specific to cytokeratins conjugated to phycoerythrin (PE); 0.0012% mouse anti-CD45 mouse monoclonal antibody conjugated to allophycocyanin (APC) in phosphate buffered saline (PBS) containing 0.5% BSA, and 0.1% sodium azide. (white cap)[1] |
| CHANGE #2 Addition: REAGENT STORAGE AND HANDLING for open container stability of the individual bottle of CellSearch Dilution Buffer | No documentation in the current Instructions for Use concerning open bottle stability. Current labeling does include: Store at 2 to 8ºC. Bring to room temperature (15 to 30ºC) before use. | Addition: "NOTE: After opening, the dilution buffer bottle (which is not a part of the reagent pack) must be stored at room temperature for up to 30 days."[2] |

| Differences | | |
|---|---|---|
| Item | Predicate | Device |
| CHANGE #3 Addition: LIMITATIONS | Requested by OIVD. | Addition: "This prognostic study does not demonstrate that any current line of therapy is any more or less effective than any other or no therapy". |
| CHANGE #4 Modification:<br>CLINICAL TRIAL RESULTS | Original Clinical Trial Results previously cleared in K031588 and K050245 | Update of Clinical Trial Results[3]: •Expanded follow-up time of patients<br>•Include shorter, separate time periods of CTC measurements after initiation of therapy |

## K. Standard/Guidance Document Referenced (if applicable):

The CellSearch™ Circulating Tumor Cell kit was developed in conformance to the following standards and guidances.

ISO 14971 Medical Devices- Application of Risk Management to Medical Devices

Guidance for Industry and FDA Staff Class II Special Controls Guidance Document: Circulating Cancer Cell Selection and Enumeration System (May 11,2004)

Guidance for Industry and FDA Staff: Use of Symbols on Labels and in Labeling of In Vitro Diagnostic Devices Intended for Professional Use (November 30, 2004)

The CellTracks Analyzer II performance data were developed in conformance to the following standards.

EP5-A NCCLS document: Evaluation of Precision Performance of Clinical Chemistry Devices

EP9-A NCCLS document: Method comparison and Bias Estimation Using Patient Samples

All requirements for these standards were met. EP9-A testing was performed using donor spiked samples rather than actual cancer patient samples.

## L. Test Principle:

Epithelial cells are immunomagnetically labeled by targeting the Epithelial Cell Adhesion Molecule (EpCAM) antigen. Anti-EpCAM monoclonal antibodies conjugated to ferrofluid particles are colloidal and, when mixed with a sample

containing the target epithelial cells, bind to the EpCAM antigen associated with the epithelial cells. After immunomagnetic selection of epithelial cells from 7.5 mL of blood, fluorescent reagents are added at this time to discriminate between the immunomagnetically selected cells. Anti-Cytokeratin – Phycoerythrin (CK-PE) stains the intracellular cytoskeleton cytokeratin proteins expressed in cells of epithelial origin, anti-CD45-Allophycocyan (CD45-APC) stains leukocytes and DAPI stains DNA present in the cell nucleus.

The processed reagent/sample mixture is dispensed by the CellTracks® AutoPrep System into a cartridge that is inserted into a MagNest® cell presentation device. The strong magnetic field of the MagNest® device causes the magnetically-labeled target cells to move to the surface of the cartridge. The cartridge is then placed on the CellTracks® Analyzer II for data acquisition and analysis. The CellTracks Analyzer II scans the entire surface of the cartridge with a series of fluorescence filters that are defined for a given assay and acquires images of PE, APC and DAPI fluorescence staining of the entire viewing surface.

After data acquisition is completed, the images are analyzed for any event where cytokeratin-PE and DAPI are within a specified space in the cartridge, i.e. indicating the possible presence of a cell with a nucleus that expresses cytokeratin. Images from each fluorescent color as well as a composite image of the cytokeratin staining (green) and the nuclear staining (purple) are presented to the user in a gallery for final cell classification. A cell is classified as a tumor cell when it its EpCAM+ (i.e., it is captured), CK+, DAPI+ and CD45-. A check mark placed by the operator next to the composite images classifies the event as a Circulating Tumor Cell (CTC) and the software tallies all the checked boxes to obtain the CTC count.

The sponsor's data demonstrate that metastatic breast cancer patients with 5 or more CTC/per 7.5 mL of blood have a significantly greater probability for shorter progression free and overall survival than patients who have fewer than 5 CTC per 7.5 mL of blood.

This test methodology is new. It is hoped that further clinical studies reported in the scientific literature will corroborate the clinical study performed by the sponsor for submission to the FDA for the predicate device.

**M. Performance Characteristics (if/when applicable):**

1. <u>Analytical performance was presented originally in K031588:</u>

   *a. Precision/Reproducibility:*

   **a. System Reproducibility with CellSearch™ Circulating Tumor Cell Control**

   Three separate CellSearch™ Circulating Tumor Cell Control samples were prepared and processed each day for over 30 days, per the long run method of

NCCLS guideline EP5-A[2]. Each single-use sample bottle contains a low and a high concentration of cells from a fixed cell line that have been pre-stained with two different fluorochromes. Summary statistics for the high and low control cells is

| | **CTC <5** | **CTC ≥5** |
|---|---|---|
| **Number of Duplicates** | 123 | 40 |
| **Mean CTC Count of Duplicates** | 0.7 | 210 |
| **Avg. Duplicate Standard Deviation** | 0.5 | 12 |
| **Avg. %CV of Duplicates** | 60% | 20% |

presented below.

**Table 1. Summary of Precision Analyses**

| | *Low* | **High** |
|---|---|---|
| N | 99 | 99 |
| Mean cell count | 48 | 969 |
| Total Precision Standard Deviation ($S_T$) % CV | 18% | 5% |

**b. System Reproducibility with Patient Samples**

A total of 163 duplicate samples were collected from 47 patients over the course of the clinical study. These samples were processed at multiple sites to determine the reproducibility of CTC measurements. The regression equation for the comparison of these 163 duplicate samples was $Y=0.98x + 0.67$, $R^2=0.99$. **Table 2** shows the summary of the data for replicates where the average of the two CTC results was <5 compared to those where the average (avg.) was ≥5.

**Table 2. Reproducibility of CTC Counts in Duplicate Samples (n=163) with an Average of <5 or ≥5 CTC per 7.5 mL of blood.**

*b. Accuracy/Recovery*:

Blood samples from a single healthy donor were pooled and five of six 7.5 mL aliquots were spiked with 5, 20, 81, 325 and 1300 cultured breast cancer cells (SK-Br-3). The sixth tube was unspiked pooled blood and served as a zero point. These samples were processed on the CellTracks® AutoPrep System with the CellSearch™ Circulating Tumor Cell Kit and CTC counts were determined on the CellTracks® Analyzer II. The experiment was repeated for four additional donors. The observed cell counts were plotted against the results of the expected cell count. The results are summarized in **Table 3**.

**Table 3. Percent Detection Estimates.**

| Expected Tumor Cell Count | Mean Observed Tumor Cell Count | Range of Percent Recovery |
|---|---|---|
| 1300 | 1215 | 91 to 95% |
| 325 | 308 | 82 to 101% |
| 81 | 85 | 80 to 136% |
| 20 | 22 | 95 to 140% |
| 5 | 7 | 120 to 200% |

To determine the overall, or least squares fit, for the comparison of the observed and expected cell counts across all the data, linear regression analysis was performed. The regression equation for these 30 samples was $Y=0.93x + 3.87$, $R^2=0.999$. The results of this study indicate that on average over the tested CTC range the recovery, as derived from regression analysis, is 93%.

Given the linear response of the tumor cell counts, one would expect the slope of the observed versus expected plot to be 1.0. However, the slope was 0.93. This is because the CellTracks® AutoPrep System with CellSearch™ CTC Kit involves the capture and fluorescent labeling of cells followed by their detection and enumeration by the CellTracks® Analyzer II. The loss of cells could therefore be attributed to one of the following possibilities; 1) the recovery of only 93% of the tumor cells spiked into 7.5mL of blood by the CellTracks® AutoPrep System, 2) the detection of only 93% of the tumor cells present in the sample chamber by the CellTracks® Analyzer II or 3) a combination of both of these sources of error.

*Linearity/assay reportable range:*

Another way to examine the previous data is to analyze it as a dilution series to evaluate test linearity. We removed the confounding variable of percent recovery by using the observed value of the original sample divided by the dilution factors to determine the expected values for the dilution series for each patient sample. Regression of all of these numbers of observed tumor cells versus the numbers of expected tumor cells yielded a slope of 1.007, an intercept of 3.0, an $r^2 = 0.99$ and $r = 0.995$. Therefore, once the percent recovery (cell loss) was factored out of the CTC values of each of the original samples, this analysis of the data demonstrated that the detection of CTC was linear over the reportable range of 0 to 1238 tumor cells.

*c. Traceability, Stability, Expected values (controls, calibrators, or methods):*

No recognized reference material or method.

*d. Detection limit:*

One CTC per 7.5 mL can be detected by the CellTracks® Analyzer II resulting in a limit of detection of 1 CTC in a cartridge. Linear regression shows that on average, 93% of CTC present in a 7.5 mL blood sample are recovered using the CellTracks® AutoPrep System (see **Recovery** section). The loss of approximately 7% of the CTC in the sample is not sufficient to reduce the limit of detection of 1 CTC.

*e. Analytical specificity:*

The CellSearch™ Circulating Tumor Cell Kit contains a ferrofluid-based capture reagent and immunofluorescent reagents. The ferrofluid reagent consists of nanoparticles with a magnetic core surrounded by a polymeric layer coated with antibodies targeting the EpCAM antigen for capturing CTC. After immunomagnetic capture and enrichment, fluorescent reagents are added for identification and enumeration of CTC. The fluorescent reagents include the following: anti-CK-Phycoerythrin (PE) specific for the intracellular protein cytokeratin (characteristic of epithelial cells), DAPI which stains the cell nucleus, and anti-CD45-Allophycocyanin (APC) specific for leukocytes

*Interfering Substances*:

SK-BR-3 cells spiked into blood samples were exposed to potential interfering substances and compared to untreated controls. Toxic levels (5 times therapeutic index) of the following cancer drugs, over-the-counter drugs, and other exogenous substances were tested: cyclophosphamide, Mitomycin C®, Procrit®, biotin, 5-fluorouracil, methotrexate, tamoxifen citrate, paclitaxel, Arimidex®, acetaminophen, acetylsalicylic acid, caffeine, dextromethorphan, Aredia®, Human Anti-Mouse Antibody (HAMA) type 1, HAMA type 2, Herceptin®, and ibuprofen. No significant differences in SK-BR-3 cell numbers were detected, indicating that these substances do not interfere with the CellSearch™ kit.

Samples spiked with toxic levels of doxorubicin resulted in aberrant staining of leukocytes as cytokeratin and CD45 dual positive cells, due to the doxorubicin being a fluorescent compound that is incorporated into nucleated cells. If seen, the staining pattern of all cells being CD45 positive and cytokeratin positive is obvious and easily identified by the operator as a known interference staining profile. If blood is drawn after the recommended 7-day washout period, following doxorubicin infusion, this interference is unlikely to be observed in clinical practice given controlled therapeutic levels and rapid drug clearance.

Potential interference from lipemia was studied by adding Intralipid to

samples to a concentration of 2.6%, which corresponds to greater than 1000 mg/dL triglyceride. Samples were lysed to simulate total hemolysis. Bilirubin at 7.4 mg/dL, HAMA 1/HAMA 2 and hematocrit from 18-60% were studied. Lipemia, hemolysis, icterus and a broad range of hematocrit values do not interfere with the CellSearch™ test. HAMA 1 and HAMA 2 also do not interfere, indicating that individuals receiving mouse Ig by parenteral routes can be tested successfully with the CellSearch™ test.

*f. Assay cut-off:*

Results are reported as the number of CTC / 7.5 mL of blood. A CTC count of 5 or more per 7.5 mL of blood is predictive of shorter progression free survival and overall survival. This cut-off was established in the 510(k) of the previous version of this assay, the predicate device, K031588.

2. Comparison studies:

*a. Method comparison with predicate device:*

Not Necessary

*b. Matrix comparison:*

Since there is only one matrix for this test. whole blood, no matrix comparison studies were performed.

3. Clinical studies:

*a. Clinical Sensitivity:*

Not applicable

*b. Clinical specificity:*

Not applicable

c. Other clinical supportive data (when a. and b. are not applicable):

Expanded follow-up time of the patient data reported in 3-4 week segments/groups was added to the CLINICAL TRIALS RESULTS section of the package insert. This data presented in the updated package insert demonstrated that a CTC result determined at any time up to 20 weeks after the first (baseline) measurement has equivalent prediction for progression free and overall survival as the first sample, which was the claim of the original predicate product. Following is the new and expanded CLINICAL TRIALS RESULTS section of the package insert:

**Metastatic Breast Cancer Patients**

A multi-center prospective, clinical trial was conducted. Results were used to determine whether the number of CTC predict disease progression and survival. Patients with measurable disease who were starting a new line of therapy were enrolled (N=177). Clinical data were analyzed on an intent-to-treat basis.

**Table 5. Patient Demographics**

| | |
|---|---|
| **Age at Baseline (Median)** | 58.5 ± 13.4 |
| **Race: White** | 153 (86%) |
| **Black** | 14 ( 8%) |
| **Hispanic** | 7 ( 4%) |
| **Unknown** | 3 ( 2%) |
| **ER/PR +** | 121 (68%) |
| **ER/PR -** | 54 (31%) |
| **Unknown** | 2 ( 1%) |
| **HER-2/neu -** | 91 (52%) |
| **HER-2/neu 1+** | 12 ( 7%) |
| **HER-2/neu 2+** | 18 (10%) |
| **HER-2/neu 3+** | 27 (15%) |
| **Unknown** | 29 (16%) |
| **Line of Therapy** | |
| 1st | 83 (47%) |
| 2nd | 25 (14%) |
| ≥ 3rd | 67 (38%) |
| Unk.* | 2 ( 1%) |
| **Type of Therapy** | |
| Hormone | 47 (26%) |
| Chemo | 87 (49%) |
| Immu/C/H | 28 (16%) |
| H / C | 10 ( 6%) |
| No Tx** | 4 ( 2%) |
| Unk.* | 1 ( 1%) |

*Unk. = Information not available
**No Tx. = No treatment information obtained

C or Chemo = Chemotherapy
H or Hormone = Hormone Therapy
I or Immuno = Immunotherapy

Baseline CTC count was determined prior to initiation of a new line of therapy. Follow-up CTC counts were determined after the initiation of therapy at approximately 3 to 4 week intervals. For the baseline analyses, Progression Free Survival (PFS) was measured from the time of the baseline blood draw to the diagnosis of progression by CT scans and/or clinical signs and symptoms, and Overall Survival (OS) was measured from the time of baseline blood draw to the time of death. For the follow-up analyses, PFS was measured from the time of the follow-up blood draw to diagnosis of progression or death, and OS was measured from the time of the follow-up blood draw to the time of death.

**CTC frequencies**

**Table 7** summarizes the total clinical study number and percentage of patients which differs from the numbers and percentages of patients for Progression Free

Survival shown on **Table 6.** Of the total patient number of 177, 23 were not evaluable at first follow-up. Of these 23 patients, ten patients died before a follow-up blood draw could be obtained, nine patients progressed prior to a follow-up blood draw, and four were lost to follow-up. Notably, each of the ten patients who died had ≥5 to extremely high CTC counts at baseline (CTC counts 9, 11, 15, 24, 111, 126, 301, 1143, 4648 and 23618). Of the 154 patients available for follow-up, 132, 99, 129, and 84 patients had a blood draw at 3-5, 6-8, 9-14, and 15-20 weeks after initiation of therapy, respectively.

**Progression Free Survival (PFS) Analysis**

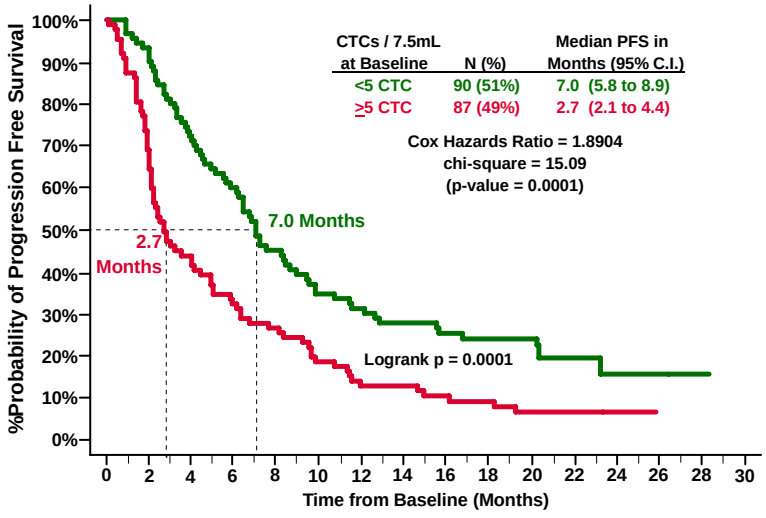
*PFS Using Baseline CTC Results*

All 177 patients had a baseline CTC test performed. For Kaplan-Meier analysis, patients were segmented into two groups based upon their CTC count at baseline:

- The Favorable group (N=90), represented in **green**, consisted of patients with <5 CTC.
- The Unfavorable group (N=87), represented in **red**, consisted of patients with ≥5 CTC.

Median PFS was 7.0 months (95% CI = 5.8 to 8.9 months) for the Favorable group and 2.7 months (95% CI = 2.1 to 4.4 months) for the Unfavorable group. The difference in PFS between the two groups is highly significant (Log-rank p=0.0001, Cox Hazards Ratio=1.8904, chi-square=15.09, p = 0.0001). These results are illustrated in **Figure 1**.

**Figure 1. PFS of Patients with < 5 or ≥ 5 CTC at Baseline (N=177).**




*PFS Using Follow-up CTC Results*

For Kaplan-Meier analysis, patients were segmented into two groups based upon their CTC count at each of the various follow-up blood draws. Both patient groups at each of the different follow-up blood draw times after initiation of therapy for PFS are illustrated in **Figure 2**. PFS times were calculated from the

time of each blood draw, and any patient showing evidence of progression prior to a particular blood draw was excluded from the analysis of that and all subsequent follow-up blood draws. **Figure 2** illustrates the ability of CTCs in patients with <5 and ≥5 CTCs 3-5 weeks, 6-8 weeks, 9-14 weeks, and 15-20 weeks after the initiation of therapy to predict time to clinical progression in 177 patients with metastatic breast cancer.

- The Favorable group represented in **olive green**, **blue**, **purple**, and **cyan** consisted of patients with <5 CTC,
- The Unfavorable group, represented in **brown**, **black**, **grey**, and **orange** consisted of patients with ≥5 CTC.

**Figure 2. PFS of Patients with <5 or ≥5 CTC at different times of Follow-Up**

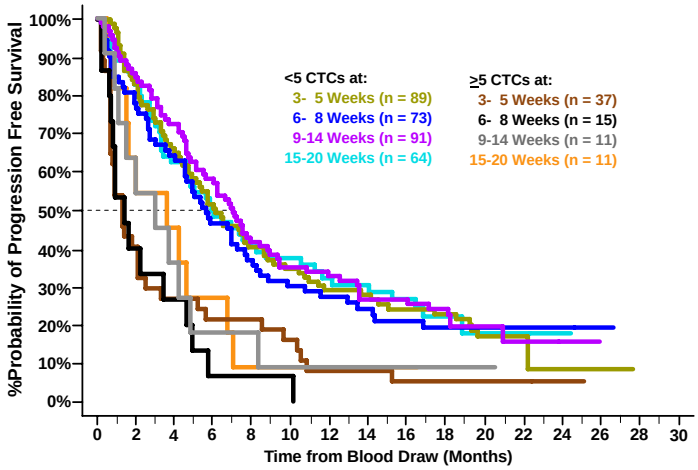



**Table 6** summarizes the results of the PFS analysis using the CTC levels and a threshold of ≥5 CTCs/7.5mL at each of the different blood draw time points.

**Table 6. Progression Free Survival (PFS) for patients with <5 or ≥5 CTC at different time points**

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|
| **Sampling Time After Tx Initiation** | **N** | **≥5 CTC** | **Median PFS in Months (95% C.I.)** | | **No PD after 6 Mo.** | | **Log-rank p-value** |
| | | | **<5 CTC** | **≥5 CTC** | **<5 CTC** | **≥5 CTC** | |
| Baseline | 177 | 87 (49%) | 7.0 (5.8 to 8.9) | 2.7 (2.1 to 4.4) | 106 (60%) | 57 (32%) | 0.0001 |
| 3-5 Weeks | 126 | 37 (29%) | 6.1 (4.7 to 8.6) | 1.3 (0.7 to 2.1) | 72 (57%) | 30 (24%) | <0.0001 |
| 6-8 Weeks | 88 | 15 (17%) | 5.6 (4.5 to 7.6) | 1.4 (0.6 to 3.4) | 53 (60%) | 24 (27%) | 0.0001 |
| 9-14 Weeks | 102 | 11 (11%) | 7.0 (5.1 to 8.8) | 3.0 (0.9 to 4.8) | 77 (75%) | 46 (45%) | 0.0134 |
| 15-20 Weeks | 75 | 11 (15%) | 6.0 (3.8 to 8.7) | 3.6 (0.7 to 6.7) | 62 (83%) | 55 (73%) | 0.0424 |

CTC = Circulating Tumor Cells, CI = Confidence Interval, PD = Progressive disease

As illustrated in **Figure 2** and **Table 6**, patients with elevated CTCs (≥5 CTC/7.5mL whole blood) at any of the time points had a much higher likelihood of rapid progression than did those with <5 CTCs. T**able 6** columns 6 & 7 show that the percentage of

patients that showed no disease progression 6 months after the initiation of therapy is higher for those patients with less than 5 CTCs as compared to those with 5 or more CTCs. T**able 6** column 4 shows the median PFS times for those patients with <5 CTCs ranged from 5.6 to 7.0 months and were substantially longer than the median PFS times for those patients with ≥5 CTCs, which ranged from 1.3 to 3.6 months (column 5). The difference in the number of patients at each time point is due to the progression of some patients prior to the blood draw and based on the number of patients sampled.

*Predictive Value of CTC Reduction or Increase on PFS*

Elapsed PFS times were calculated from the baseline blood draw. For Kaplan-Meier analysis, patients were segmented into four groups based upon their CTC counts:

- Group 1 (**green** curve), 83 (47%) patients with <5 CTCs at all blood draw time points;
- Group 2 (**blue** curve), 38 (21%) patients with ≥5 CTCs prior to the initiation of therapy but who had decreased to <5 CTCs at the time of their last blood draw;
- Group 3 (**orange** curve), 17 (10%) patients with <5 CTCs prior to the initiation of therapy who increased to ≥5 CTCs at the time of their last blood draw;
- Group 4 (**red** curve), 39 (22%) patients with ≥5 CTCs at all blood draw time points.

**Figure 3. A Reduction in CTC Below 5 After the Initiation of Therapy Predicts Longer PFS**

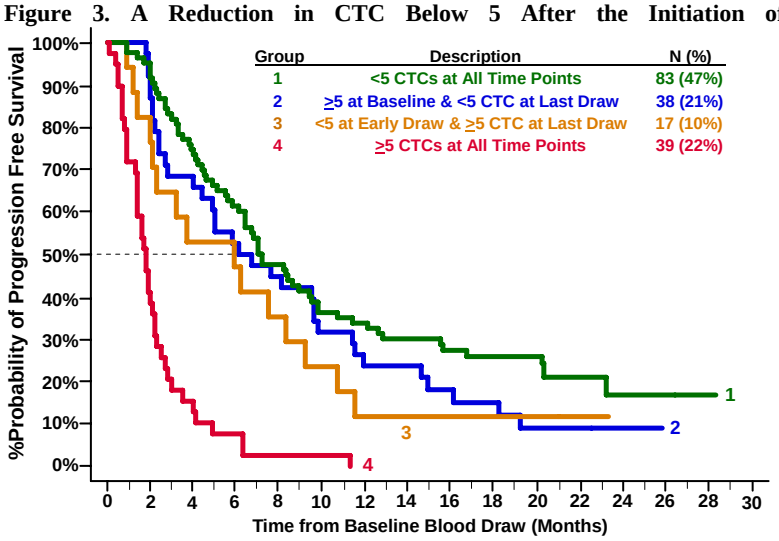



**Figure 3** shows that patients with ≥5 CTCs at all time points (Group 4) had the shortest median PFS (1.8 months, 95% CI = 1.4 - 2.2), which was significantly different compared to the median PFS of Group 3 (median PFS = 5.9 months, 95% CI = 2.0 - 9.2, log-rank p=0.0004), Group 2 (median PFS = 6.1 months, 95% CI = 4.0 – 9.6, log-rank p<0.0001), and Group 1 (median PFS = 7.2 months, 95% CI = 5.8 – 9.5, log-rank p<0.0001). Differences between the curves for the other groups in this figure were not significant (log-rank p>0.1280).

**Overall Survival (OS) Analysis**

*OS Analysis Using Baseline CTC Results*

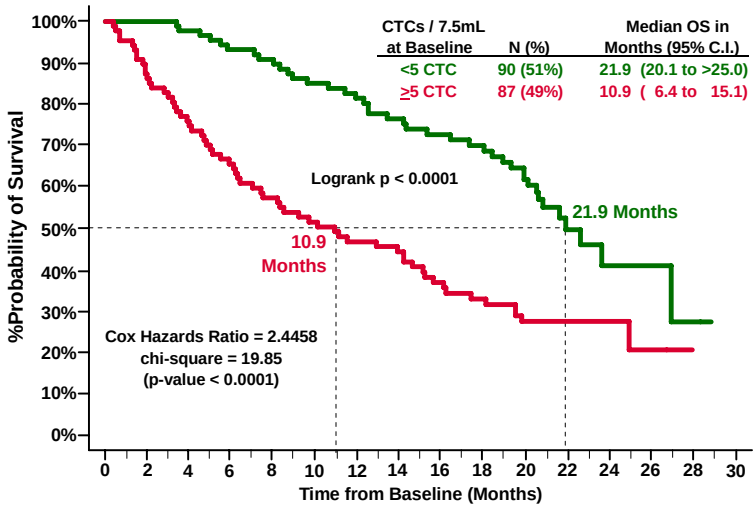
Death occurred in 66 (37%) of the 177 patients during this study. At the time of these analyses, seventeen (19%) of 90 patients from Favorable group (<5 CTC at baseline) compared to 49 (56%) of 87 from Unfavorable group (≥5 CTC at baseline) had died.

For Kaplan-Meier analysis, patients were segmented into two groups based upon their CTC count at baseline:

- The Favorable group (N=90), represented in **green**, consisted of patients with <5 CTC.
- The Unfavorable group (N=87), represented in **red**, consisted of patients with ≥5 CTC.

Median OS was 21.9 months (95% CI = 20.1 to >25.0 months) for the Favorable group and 10.9 months (95% CI = 6.4 to 15.1 months) for the Unfavorable group. The OS difference between the two groups is highly significant (Log-rank p<0.0001, Cox Hazards Ratio=2.4458, chi-square=19.85, p< 0.0001). These results are illustrated in **Figure 4**.

**Figure 4. OS of Patients with < 5 or ≥ 5 CTC at Baseline (N=177).**




*OS Using Follow-up CTC Results*

The Kaplan-Meier analyses of both patient groups at each of the different follow-up blood draw times after initiation of therapy are illustrated in **Figure 5**. This figure illustrates the ability of CTCs in patients with <5 and ≥5 CTCs 3-5 weeks, 6-8 weeks, 9-14 weeks, and 15-20 weeks after the initiation of therapy to predict time to death in 177 patients with metastatic breast cancer. OS times were calculated from the time of each blood draw.

- The Favorable group represented in **olive green**, **blue**, **purple**, and **cyan** consisted of patients with <5 CTC,

- The Unfavorable group, represented in **brown**, **black**, **grey**, and **orange** consisted of patients with ≥5 CTC.

**Figure 5. OS of Patients with <5 or ≥5 CTC at different times of Follow-Up.**

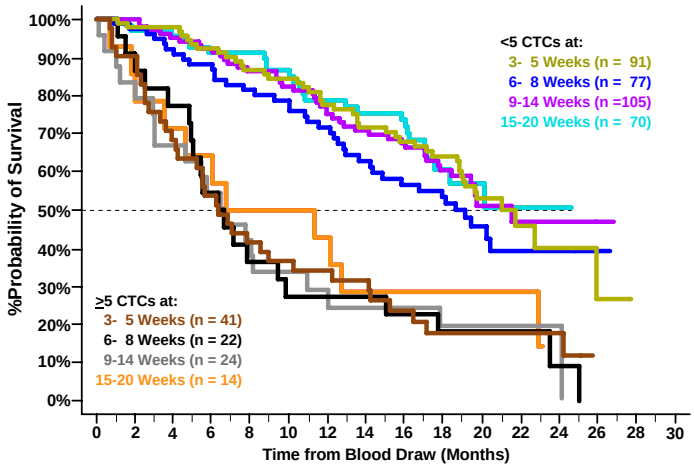



**Table 7** summarizes the results of the OS analysis using the CTC levels and a threshold of ≥5 CTCs/7.5mL at each of the different blood draw time points. **Table 7** columns 6 & 7 show that the percentage of patients that are alive 12 months after the initiation of therapy is higher for those patients with less than 5 CTCs as compared to those with 5 or more CTCs.

**Table 7. Overall Survival (OS) for patients with <5 or ≥5 CTC at different time points**

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|
| **Sampling Time After Tx Initiation** | **N** | **≥5 CTC** | **Median OS in Months (95% C.I.)** | | **Alive after 12 Months** | | **Log-rank p-value** |
| | | | **<5 CTC** | **≥5 CTC** | **<5 CTC** | **≥5 CTC** | |
| Baseline | 177 | 87 (49%) | 21.9 (20.1 to >25) | 10.9 (6.4 to 15.1) | 145 (82%) | 83 (47%) | <0.0001 |
| 3-5 Weeks | 132 | 41 (31%) | 21.0 (18.8 to >25) | 6.3 (4.1 to 10.2) | 107 (81%) | 45 (34%) | <0.0001 |
| 6-8 Weeks | 99 | 22 (22%) | 18.6 (14.2 to >25) | 6.3 (4.8 to 9.8) | 76 (77%) | 27 (27%) | 0.0001 |
| 9-14 Weeks | 129 | 25 (19%) | 21.5 (17.8 to >25) | 6.6 (3.0 to 10.9) | 110 (85%) | 43 (33%) | <0.0001 |
| 15-20 Weeks | 84 | 14 (17%) | >25 (17.1 to >25) | 6.7 (2.0 to 22.9) | 76 (91%) | 42 (50%) | 0.0013 |

*Predictive Value of CTC Reduction or Increase on OS*

Elapsed OS times were calculated from the baseline blood draw. For Kaplan-Meier analysis, patients were segmented into four groups based on their CTC counts:

- Group 1 (**green** curve), 83 (47%) patients with <5 CTCs at all blood draw time points;
- Group 2 (**blue** curve), 38 (21%) patients with ≥5 CTCs prior to the initiation of therapy but who had decreased to <5 CTCs at the time of their last blood draw;
- Group 3 (**orange** curve), 17 (10%) patients with <5 CTCs prior to the initiation of therapy who increased to ≥5 CTCs at the time of their last blood draw;
- Group 4 (**red** curve), 39 (22%) patients with ≥5 CTCs at all blood draw time points.

**Figure 6. A Reduction in CTC Below 5 After the Initiation of Therapy Predicts Longer OS whereas an Increase in CTC Count to 5 or above Predicts a Shorter OS**

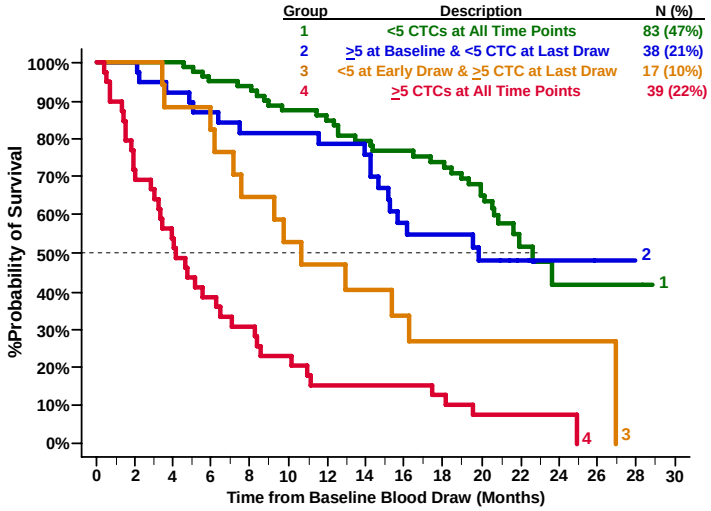



**Figure 6** shows that patients who exceed the threshold of 5 CTCs at any point after the initiation of therapy are at a significantly higher risk of dying sooner. Patients with ≥5 CTCs at all time points (Group 4) had the shortest median OS (4.1 months, 95% CI = 2.8 - 6.4), which was significantly different compared to the median OS of Group 3 (median OS = 10.6 months, 95% CI = 6.1 - 16.2, log-rank p=0.0051), Group 2 (median OS = 19.8 months, 95% CI = 14.6 - >25, log-rank p<0.0001), and Group 1 (median OS = 22.6 months, 95% CI = 20.5 - >25, log-rank p<0.0001). Differences between Groups 3 and 2 (p=0.0397) and Groups 3 and 1 (log-rank p=0.0014) were also significant. **Figure 6** also shows that patients who have ≥5 CTCs at baseline but eventually decrease to <5 CTCs after the initiation of therapy have approximately the same risk of death as those patients who never exceed the 5 CTC threshold.

As illustrated in **Figure 6** and **Table 7** in columns 4 & 5, patients with ≥5 CTCs at any of the time points had a much higher likelihood of dying sooner than did those with <5 CTCs. The median OS times for those patients with <5 CTCs ranged from 18.6 to >25.0 months and were substantially longer than the median OS times for those patients with ≥5 CTCs, which ranged from 6.3 to 10.9 months.

## Univariate Cox Regression Analysis

**Table 8** shows the Cox Regression univariate analysis

**Table 8: Univariate Analysis**

| Parameter | Categories | | PFS Risk from Baseline | | | | OS Risk from Baseline | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Pos | Neg | HR | p-value | $chi^2$ | # of Pts | HR | p-value | $chi^2$ | # of Pts |
| Age at Baseline Blood Draw | Age in Years | | 0.9917 | 0.1508 | 2.06 | 175 | 0.9910 | 0.2112 | 1.56 | 175 |
| Stage at Primary Diagnosis | 4 vs. 3 vs. 2 vs. 1 | | 0.9636 | 0.6991 | 0.15 | 164 | 1.0131 | 0.9118 | 0.01 | 164 |
| ER/PR | Pos | Neg | 0.8484 | 0.3497 | 0.87 | 175 | 0.5388 | 0.0038 | 8.39 | 175 |
| Her-2/neu | 3+ vs. 2+ vs. 1+ vs. 0 | | 0.9060 | 0.1770 | 1.82 | 148 | 0.9076 | 0.2791 | 1.17 | 148 |
| ECOG Status | 2 vs. 1 vs. 0 | | 1.1297 | 0.3319 | 0.94 | 172 | 1.5980 | 0.0018 | 9.75 | 172 |
| Time to Metastasis | Time in Years | | 0.9709 | 0.0367 | 4.36 | 175 | 0.9431 | 0.0031 | 8.75 | 175 |
| Line of Therapy | ≥2nd | 1st | 1.5358 | 0.0091 | 6.81 | 175 | 1.8666 | 0.0019 | 9.64 | 175 |
| Type of Therapy | C / O | Horm/ Immun | 1.8141 | 0.0009 | 10.95 | 172 | 3.7881 | 0.0000 | 28.22 | 172 |
| Baseline CTC Number | ≥5 | <5 | 1.8904 | 0.0001 | 15.09 | 177 | 2.4459 | 0.0000 | 19.85 | 177 |
| 3 - 5 Week CTC Number | ≥5 | <5 | 2.4136 | 0.0000 | 17.29 | 132 | 3.3816 | 0.0000 | 24.62 | 132 |
| 6 - 8 Week CTC Number | ≥5 | <5 | 3.5709 | 0.0000 | 19.56 | 99 | 2.7871 | 0.0006 | 11.72 | 99 |
| 9 - 14 Week CTC Number | ≥5 | <5 | 2.9300 | 0.0001 | 16.15 | 129 | 3.8547 | 0.0000 | 19.78 | 129 |
| 15 - 20 Week CTC Number | ≥5 | <5 | 1.9910 | 0.0401 | 4.21 | 84 | 3.0735 | 0.0047 | 7.99 | 84 |

**Pos** – Positive; **Neg** – Negative; **Pts** – Patients; **C/O** – Chemo or Other; **Horm/Immun** – Hormonal/ Immuno

## Multivariate Cox Regression Analysis

The following parameters were evaluated using multivariate Cox regression analysis, with the SAS PROC PHREG (regression Analysis of Survival Data Based on the Cox Proportional Hazards Model), stepwise selection process to evaluate association with PFS and OS: patient age (continuous), stage of disease at diagnosis (I-IV), time to metastasis (continuous), ECOG status before initiation of a new line of therapy (0-2), ER/PR status (+/-), HER2/neu status (0-3), line of therapy ($\geq 2^{nd}$ or $1^{st}$), type of therapy (chemo/other or hormonal/immuno), baseline CTC count ($\geq$5 or <5 CTC/7.5mL), and $1^{st}$ follow-up CTC count ($\geq$5 or <5 CTC/7.5mL). A stringency level (p-value) of 0.05 was used to both include and exclude parameters in the multivariate analyses. Results for each parameter that demonstrated a statistically significant correlation to PFS and OS are summarized in **Tables 9** and **10,** respectively. CTC number was the strongest predictor of PFS and OS.

## Table 9. Multivariate Cox Analysis: Stepwise Cox Regression for Prediction of PFS

| Parameter | Categories | | PFS Risk from Baseline | | | |
|---|---|---|---|---|---|---|
| | Positive | Negative | HR | p-value | $chi^2$ | # of Patients |
| Line of Therapy | ≥2nd | 1st | 1.637 | 0.003 | 28.74 | 172 |
| Type of Therapy | Chemo/Other | Hormonal | 1.635 | 0.010 | | |
| Baseline CTC Number | ≥5 | <5 | 1.709 | 0.001 | | |

| Parameter | Categories | | PFS Risk from Baseline | | | |
|---|---|---|---|---|---|---|
| | Positive | Negative | HR | p-value | $chi^2$ | # of Patients |
| Line of Therapy | ≥2nd | 1st | 1.600 | 0.015 | 23.19 | 132 |
| 3 - 5 Week CTC Number | ≥5 | <5 | 2.277 | 0.000 | | |

| Parameter | Categories | | PFS Risk from Baseline | | | |
|---|---|---|---|---|---|---|
| | Positive | Negative | HR | p-value | $chi^2$ | # of Patients |
| Stage at Primary Diagnosis | 4 vs. 3 vs. 2 vs. 1 | | 0.706 | 0.037 | 30.68 | 83 |
| Her-2/neu | 3+ vs. 2+ vs. 1+ vs. 0 | | 0.796 | 0.039 | | |
| Time to Metastasis | Time in Years | | 0.862 | 0.007 | | |
| Type of Therapy | Chemo/Other | Hormonal | 2.387 | 0.017 | | |
| 6 - 8 Week CTC Number | ≥5 | <5 | 3.089 | 0.000 | | |

| Parameter | Categories | | PFS Risk from Baseline | | | |
|---|---|---|---|---|---|---|
| | Positive | Negative | HR | p-value | $chi^2$ | # of Patients |
| 9 - 14 Week CTC Number | ≥5 | <5 | 2.930 | 0.000 | 16.15 | 129 |

| Parameter | Categories | | PFS Risk from Baseline | | | |
|---|---|---|---|---|---|---|
| | Positive | Negative | HR | p-value | $chi^2$ | # of Patients |
| Time to Metastasis | Time in Years | | 0.938 | 0.029 | 22.24 | 84 |
| Line of Therapy | ≥2nd | 1st | 2.141 | 0.004 | | |
| Type of Therapy | Chemo/Other | Hormonal | 1.773 | 0.049 | | |
| 15 - 20 Week CTC Number | ≥5 | <5 | 2.170 | 0.019 | | |

**Table 10. Multivariate Cox Analysis: Stepwise Cox Regression for Prediction of OS**

| Parameter | Categories | | OS Risk from Baseline | | | |
|---|---|---|---|---|---|---|
| | Positive | Negative | HR | p-value | $chi^2$ | # of Patients |
| ECOG Status | 2 vs. 1 vs. 0 | | 1.686 | 0.000 | 75.27 | 170 |
| Time to Metastasis | Time in Years | | 0.942 | 0.021 | | |
| Line of Therapy | ≥2nd | 1st | 2.362 | 0.000 | | |
| Type of Therapy | Chemo/Other | Hormonal | 3.562 | 0.000 | | |
| Baseline CTC Number | ≥5 | <5 | 2.314 | 0.000 | | |

| Parameter | Categories | | OS Risk from Baseline | | | |
|---|---|---|---|---|---|---|
| | Positive | Negative | HR | p-value | $chi^2$ | # of Patients |
| ECOG Status | 2 vs. 1 vs. 0 | | 1.818 | 0.000 | 52.79 | 130 |
| Line of Therapy | ≥2nd | 1st | 1.792 | 0.016 | | |
| Type of Therapy | Chemo/Other | Hormonal | 3.240 | 0.000 | | |
| 3 - 5 Week CTC Number | ≥5 | <5 | 2.672 | 0.000 | | |

| Parameter | Categories | | OS Risk from Baseline | | | |
|---|---|---|---|---|---|---|
| | Positive | Negative | HR | p-value | $chi^2$ | # of Patients |
| Age at Baseline Blood Draw | Age in Years | | 1.023 | 0.030 | 56.28 | 99 |
| Time to Metastasis | Time in Years | | 0.867 | 0.005 | | |
| Line of Therapy | ≥2nd | 1st | 2.825 | 0.000 | | |
| Type of Therapy | Chemo/Other | Hormonal | 15.176 | 0.000 | | |
| 6 - 8 Week CTC Number | ≥5 | <5 | 3.789 | 0.000 | | |

| Parameter | Categories | | OS Risk from Baseline | | | |
|---|---|---|---|---|---|---|
| | Positive | Negative | HR | p-value | $chi^2$ | # of Patients |
| ER/PR | Positive | Negative | 0.445 | 0.007 | 63.28 | 129 |
| ECOG Status | 2 vs. 1 vs. 0 | | 1.611 | 0.013 | | |
| Line of Therapy | ≥2nd | 1st | 2.215 | 0.004 | | |
| Type of Therapy | Chemo/Other | Hormonal | 4.004 | 0.000 | | |
| 9 - 14 Week CTC Number | ≥5 | <5 | 4.885 | 0.000 | | |

| Parameter | Categories | | OS Risk from Baseline | | | |
|---|---|---|---|---|---|---|
| | Positive | Negative | HR | p-value | $chi^2$ | # of Patients |
| Time to Metastasis | Time in Years | | 0.873 | 0.015 | 38.65 | 84 |
| Line of Therapy | ≥2nd | 1st | 3.799 | 0.001 | | |

4. Clinical cut-off**:**

Results are reported as the number of CTC / 7.5 mL of blood. A CTC count of 5 or more per 7.5 mL of blood is predictive of shorter progression free survival and overall survival. This cut-off was established in the 510(k) of the previous version of this assay, the predicate device, K031588.

5. Expected values/Reference range:

**EXPECTED VALUES**

The EXPECTED VALUES were determined with a previous version of this test, the original version, as presented in K031588. The present version of the test was demonstrated to be substantially equivalent to that in K031588 in K050245.

*Healthy volunteers, non-malignant breast disease, non-malignant other disease*

Single point CTC analyses were performed on control groups of 145 healthy volunteers, 101 women with non-malignant breast disease, and 99 women with other non-malignant diseases.

Epithelial cells are not expected to be present in the peripheral blood of healthy individuals. Of the 345 total samples from healthy volunteers and women with non-malignant disease, only one subject had more than 5 CTC/7.5 mL. The results are presented in **Table 1**.

**Results:**

**Table 7. Control Subjects**

| Category | N | Mean # CTC | SD | # Patients with ≥ 5 CTC | Min.* | Max.* |
|---|---|---|---|---|---|---|
| **Healthy** | 145 | 0.1 | 0.2 | 0 | 0 | 1 |
| **Non-malignant breast disease** | 101 | 0.2 | 1.2 | 1 | 0 | 12 |
| **Non-malignant other disease** | 99 | 0.1 | 0.4 | 0 | 0 | 3 |

* NCCLS Guideline C28-A2[3]

## N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

## O. Conclusion:

1. The submitted information in this premarket notification is complete and supports a substantial equivalence decision.