**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION**
**DECISION SUMMARY**

**A. 510(k) Number:**
k071729

**B. Purpose for Submission:**
To expand the indication for use of the CellSearch™ CTC Assay for use with colon cancer as well as the previously cleared breast cancer.

**C. Measurand:**
EpCam, Cytokeratins 8, 18 and/or 19, and CD45

**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
2. Classification:
   Class II
3. Product code:
   NQI system, immunomagnetic, circulating cancer cell, enumeration
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 or metastatic colon cancer. The test is to be used as an aid in the monitoring of patients with metastatic breast cancer or metastatic colon cancer. Serial testing for CTC should be used in conjunction with other clinical methods for monitoring breast cancer and colon cancer. Evaluation of CTC at any time during the course of disease allows assessment of patient prognosis of 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 (k040077) and the CellTracks® Analyzer II. (k050145 and k060110) 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 consists of anti-EpCAM Ferrofluid (mouse monoclonal antibody (mAB) to EpCAM conjugated-magnetic nanoparticles in buffer with BSA and ProClin 300), staining reagent (phycoerythrin (PE)-conjugated mouse anti-cytokeratins mAB to and allophycocyanin (APC)-conjugated mouse anti-CD45 mAB in buffer with BSA and sodium azide), nucleic acid dye (4', 6-diamidino-2-phenylindole, dihydrochloride and ProClin 300),capture enhancing reagent, permeabilization reagent, cell fixative, dilution buffer, conical tubes and caps and cartridges and cartridge plugs.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):
   The CellSearch™ Circulating Tumor Cell Kit (for breast cancer only)
2. Predicate 510(k) number(s):
   k062013
3. Comparison with predicate:

Similarities

| | **Device** | **Predicate** |
|---|---|---|
| **Manufacturer** | Veridex, LLC. Warren, NJ | Same |
| **Intended Use** | The CellSearch™ Circulating Tumor Cell Kit (CellSearch) 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. | Same |
| **Sample Type** | Whole blood | Same |
| **Instrumentation and peripherals Used** | The CellSearch system includes: CellSave Preservative Tubes, the CellTracks® AutoPrep® System, the CellTracks® Analyzer II or the CellSpotter® Analyzer, and the CellSearch™ Circulating Tumor Cell Control Kit. | Same |
| **Measurand** | CTCs positive for EpCam, Cytokeratins 8, 18 and/or 19 | Same |
| **Assay Type** | Cellular | Same |

| | Device | Predicate |
|---|---|---|
| **Technology** | Immunomagnetic capture, enrichment and detection | Same |
| **Fluorescent reagents** | Anti-CK-PE, DAPI, and anti-CD45-APC | Same |
| **Recognition of positive cells** | Visual recognition through fluorescent signal | Same |
| **Means of measurement** | Fluorescent cell counting compared to cutoff value | Same |
| **Cutoff for Progression/Survival** | Crossing of one threshold value at any time point | Same |

Differences

| | New Device | Predicate) |
|---|---|---|
| **Indication for Use** | Metastatic colon cancer or breast cancer | Metastatic breast cancer only |
| **Clinical Performance Characteristics** | Metastatic colon cancer patients studied longitudinally | Metastatic breast cancer patients studied longitudinally. Data found in the following 510(k) submissions: k031588, k050245, k052191, and k062013. |

**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)

"Guidance Document for the Submission of Tumor Associated Antigens Premarket Notifications, [510(k)], to FDA to Guide Manufacturers

**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.

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

1. Analytical performance was presented originally in K031588:

*a. Precision/Reproducibility:*

**i. 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 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% |

**ii. System Reproducibility with Patient Samples**

A total of 163 duplicate blood samples were collected from 47 metastatic breast cancer 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. **Figure 1** shows a scatter plot of the duplicate CTC results in blood from

MBC patients plotted on a logarithmic scale, with the threshold of 5 CTC indicated by the dashed lines.

**Figure 1. Reproducibility of CTC Counts in Duplicate MBC Samples (n=163) with Average of <5 or ≥5 CTC per 7.5 mL of blood.**

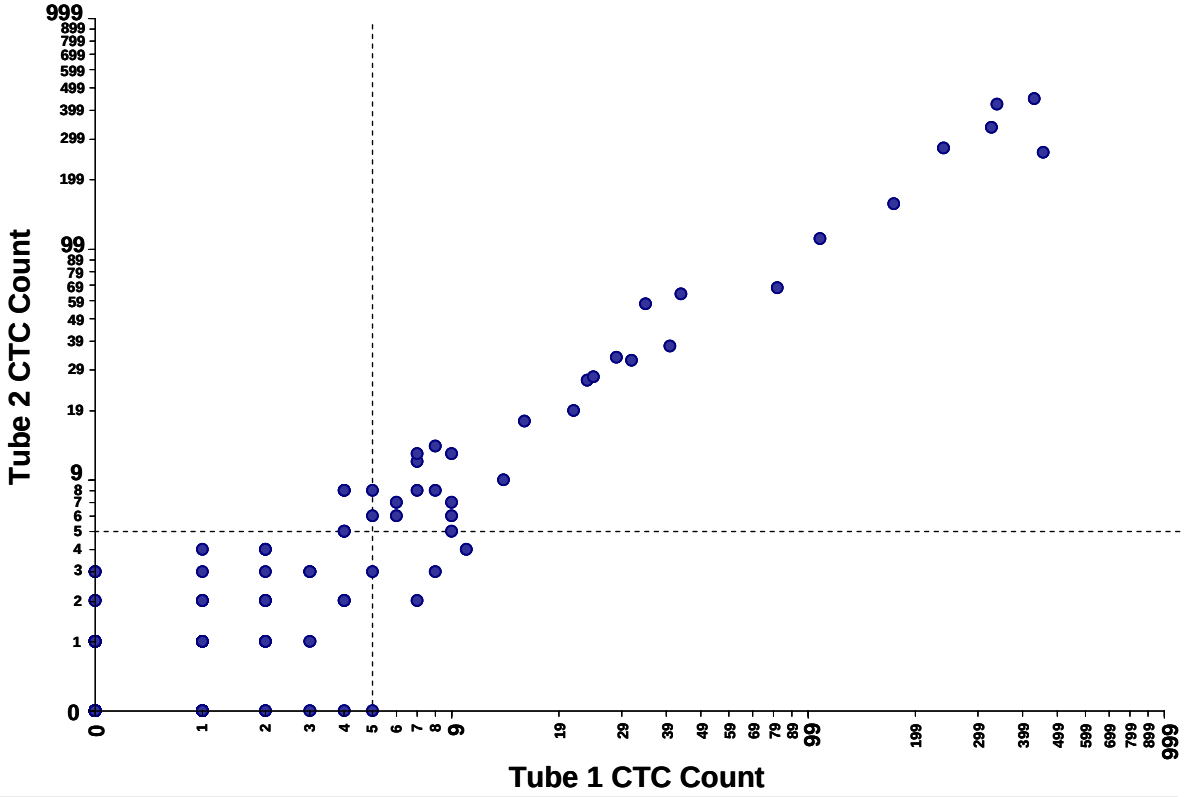



*Figure 2 Note: There may be more than one point superimposed over another. For example, on this plot, there are 50 instances (31%) where both tubes had 0 CTC, 18 instances (11%) where Tube 1 had 0 CTC and Tube 2 had 1 CTC, and another 18 instances (11%) where Tube 1 had 1 CTC and Tube 2 had 0 CTC.*

**Metastatic Colorectal Cancer (MCRC)**

A total 1,627 duplicate blood samples were collected from 430 MCRC 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 1,627 duplicate samples was $Y=0.98x + 0.18$, $R^2=0.96$. **Figure 2** shows a scatter plot of the duplicate CTC results in blood from MCRC patients plotted on a logarithmic scale, with the threshold of 3 CTC indicated by the dashed lines.

**Figure 2. Reproducibility of CTC Counts in Duplicate MCRC Samples (n=1627) with Average of <3 or ≥3 CTC per 7.5 mL of blood.**

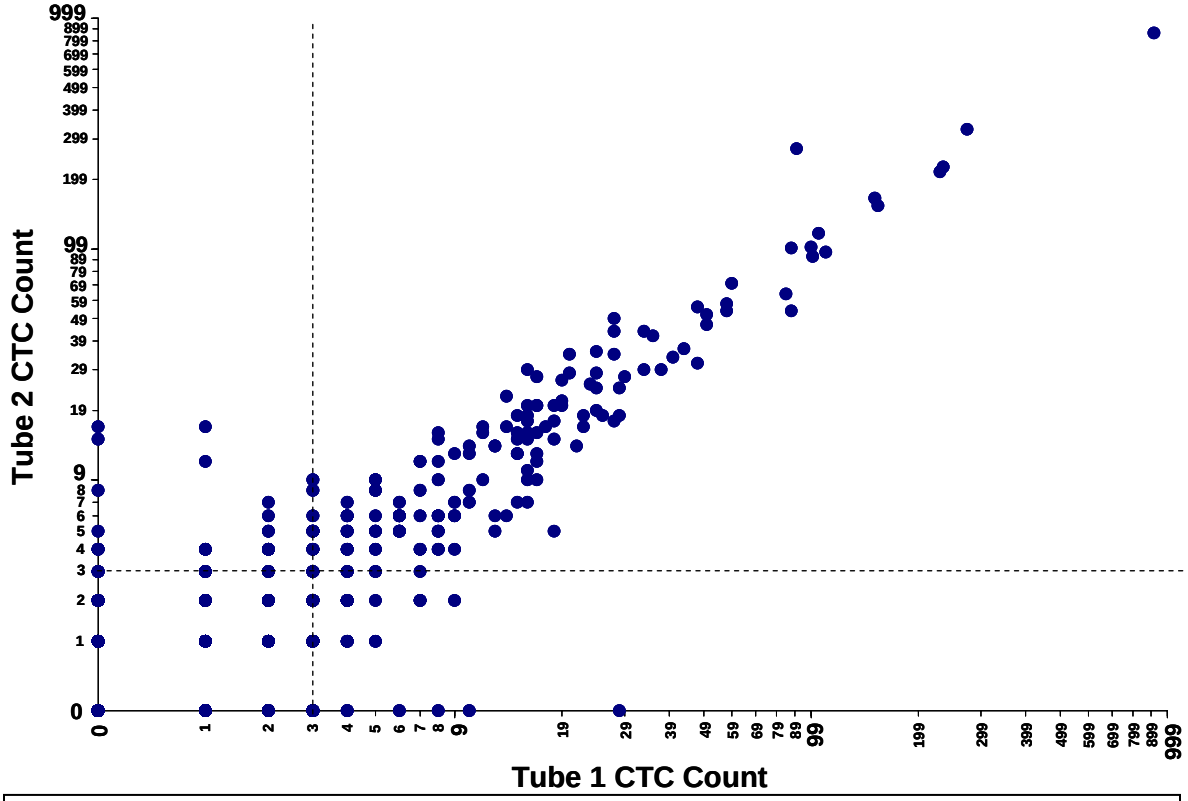



*Figure 3 Note: There may be more than one point superimposed over another. For example, on this plot, there are 975 instances (60%) where both tubes had 0 CTC, 116 instances (7%) where Tube 1 had 0 CTC and Tube 2 had 1 CTC, and another 109 instances (7%) where Tube 1 had 1 CTC and Tube 2 had 0 CTC.*

*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 2**.

**Table 2. 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.

*b. Linearity/assay reportable range:*

Another way to examine the previous data is to analyze it as a dilution series to evaluate test linearity. The confounding variable of percent recovery was removed 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, 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 3 or more per 7.5 mL of blood is predictive of shorter progression free survival and overall survival for colorectal cancer.

2. Comparison studies:

*a. Method comparison with predicate device:*
Direct comparison to the predicate device is not feasible since the patient population used by the two tests is different (metastatic breast cancer vs. metastatic colon cancer) rather, clinical studies are necessary to establish the new indication for use. These were performed by the sponsor.

*b. Matrix comparison:*
Since there is only one matrix for this test, i.e. 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):*

**CLINICAL STUDIES TO SUPPORT USE OF VERIDEX CTC TEST WITH METASTATIC COLON CANCER (MCRC) PATIENTS**

A multi-center prospective, clinical trial was conducted to determine whether the number of CTC predicted disease progression and survival. Metastatic colorectal cancer patients with measurable (N=430) disease starting a new line of therapy were enrolled. Clinical data were analyzed on an intent-to-treat basis. Patient demographic information is presented in **Table 3.**

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.

**Table 3: MCRC Patient Demographics**

| Category | Description of Categories | N=430 Patients |
|---|---|---|
| **Age at Baseline (in years)** | **Mean ± Std. Deviation (Median)** | 63.0 ± 12.6 (64) |
| **Years to Metastasis** | **Mean ± Std. Deviation (Median)** | 0.9 ± 1.4 (0.1) |
| | | **Number of Subjects (% of total)** |
| **Gender** | **Female**<br>**Male** | 192 (45%)<br>238 (55%) |
| **Race** | **White**<br>**Black**<br>**Other**<br>**Unknown** | 305 (71%)<br>44 (10%)<br>12 ( 3%)<br>69 (16%) |
| **Baseline ECOG Score** | **0**<br>**1**<br>**2**<br>**Unknown** | 196 (46%)<br>187 (43%)<br>31 ( 7%)<br>16 ( 4%) |
| **Tumor Type at Primary Diagnosis** | **Colon**<br>**Rectal**<br>**Colorectal**<br>**Unknown** | 292 (68%)<br>71 (17%)<br>66 (15%)<br>1 ( 0%) |

| | | |
|---|---|---|
| **Stage at Primary Diagnosis** | **1** | 12 ( 3%) |
| | **2** | 45 (11%) |
| | **3** | 118 (27%) |
| | **4** | 232 (54%) |
| | **Unknown** | 23 ( 5%) |
| **Liver Metastasis** | **No** | 117 (27%) |
| | **Yes** | 313 (73%) |
| **Line of Therapy** | **1st Line** | 309 (72%) |
| | **2nd Line** | 95 (22%) |
| | **3rd Line** | 26 ( 6%) |
| **Type of Therapy** | **Bevacizumab** | 243 (56%) |
| | **Irinotecan** | 103 (24%) |
| | **Oxaliplatin** | 253 (59%) |
| | **Unknown** | 25 ( 6%) |

1.1 CTC frequencies

Of the total number of 430 MCRC patients, 9 had a baseline blood draw and no follow-up blood draws. Of these 9 patients, four died before a follow-up blood draw could be obtained, two were taken off their therapy due to treatment related toxicity, one patient had surgery to remove their measurable disease, one patient refused further treatment, and one patient refused any further blood draws. Of the remaining patients, 362, 342, 321, and 211 had follow-up blood draws 1-2 weeks, 3-5 weeks, 6-12 weeks, and 13-20 weeks after the initiation of therapy, respectively. The difference in the number of patients evaluable for PFS and OS at each time point is due to the progression of some patients prior to the blood draw, while the difference in the number of patients at each time point is due to the number of patients with blood draws and evaluable CTC results.

**Table 4** shows the numbers of patients at each time point excluded from the PFS, OS, or PFS & OS analyses and the reasons for their exclusion.

**Table 4: Exclusions from Progression Free and Overall Survival Analyses**

| Blood Draw Timing | Reasons for Exclusion of MCRC Patients from Analyses: | | | | | | Total # of MCRC Patients Evaluable: | |
|---|---|---|---|---|---|---|---|---|
| | PFS & OS | | | | PFS Only | OS Only | | |
| | Blood Not Drawn | Blood Drawn 1-7 days after administration of therapy | No Follow-up Beyond Date of Blood Draw | Non-Evaluable CTC Results | Blood drawn after date of disease progression | No Follow-up Beyond Date of Blood Draw | PFS | OS |
| Baseline | 1 | 11 | 0 | 5 | 0 | 0 | 413 | 413 |
| 1-2 Weeks | 68 | 0 | 0 | 5 | 1 | 0 | 356 | 357 |
| 3-5 Weeks | 88 | 0 | 1 | 8 | 4 | 0 | 329 | 333 |
| 6-12 Weeks | 109 | 0 | 4 | 7 | 26 | 0 | 284 | 310 |
| 13-20 Weeks | 219 | 0 | 9 | 8 | 14 | 1 | 180 | 193 |

The CTC results obtained from the follow-up blood draws at 1-2 weeks, 3-5 weeks, 6-12 weeks, and 13-20 weeks after the initiation of therapy were classified as being favorable (<3 CTC) or unfavorable (≥3 CTC). If more than one CTC result was obtained within any of the designated follow-up time points, the CTC result from the blood draw furthest from the baseline blood draw was used.
**Table 6** summarizes the total number of MCRC patients and percentage of patients with unfavorable CTC in the clinical trial that differs from the numbers and percentages of patients for Progression Free Survival shown in **Table 5**.

1.2 Progression Free Survival (PFS) Analysis of MCRC Patients

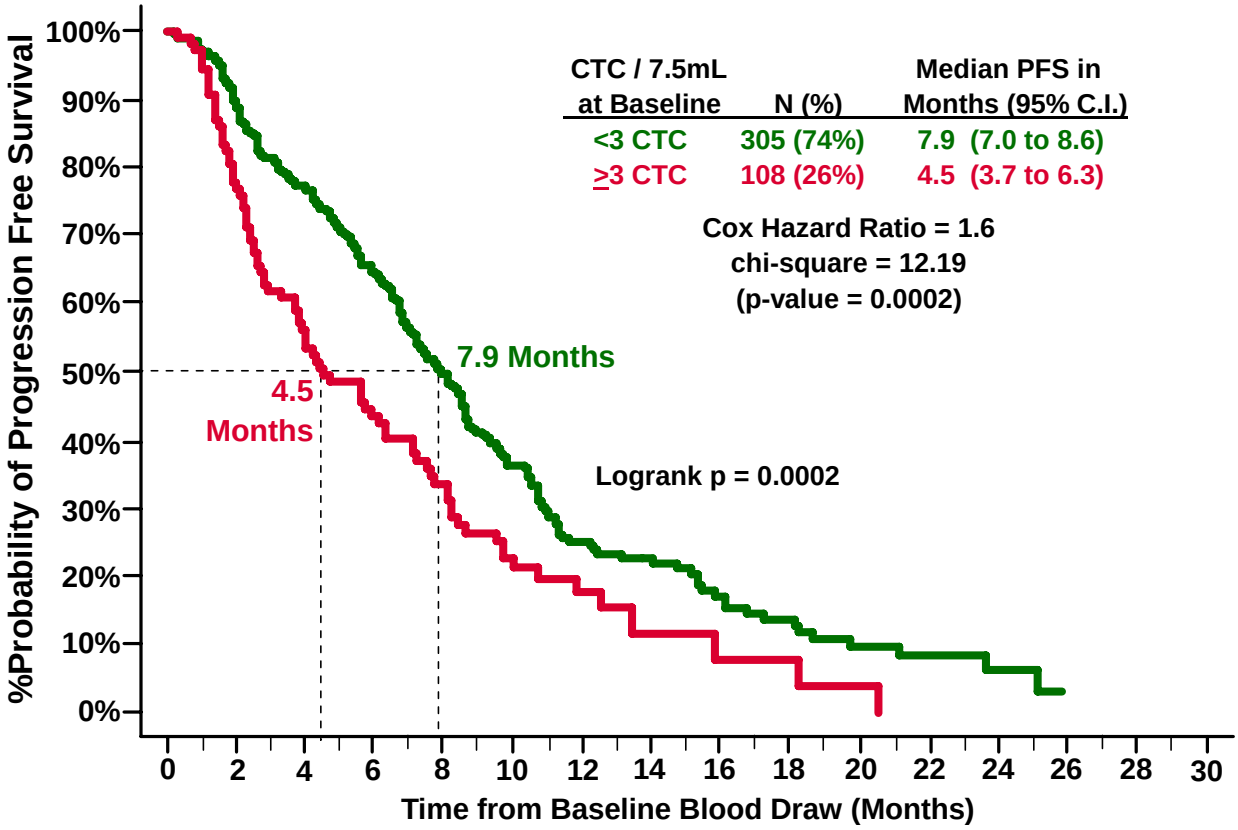
## PFS Using Baseline CTC Results

413 of the 430 MCRC patients had a baseline CTC result available. For Kaplan-Meier analysis, patients were segmented into two groups based upon their CTC count at baseline:

- The Favorable group (N=305), represented in **green**, consisted of patients with <3 CTC.
- The Unfavorable group (N=108), represented in **red**, consisted of patients with ≥3 CTC.

Median PFS was significantly longer in the Favorable group compared to the Unfavorable group (7.9 vs 4.5 months, respectively). These results are illustrated in **Figure 3** or **Table 5**.

**Figure 3: PFS of MCRC Patients with < 3 or ≥ 3 CTC at Baseline (N=413).**




## 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 4**. 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 4** illustrates the ability of CTC in MCRC patients with <3 and ≥3 CTC 1-2 weeks, 3-5 weeks, 6-12 weeks, and 13-20 weeks after the initiation of therapy to predict PFS.

- The Favorable group, represented in **olive green**, **blue**, **purple**, and **cyan**, consisted of patients with <3 CTC,
- The Unfavorable group, represented in **brown**, **black**, **grey**, and **orange**, consisted of patients with ≥3 CTC.

**Figure 4: PFS of MCRC Patients with < 3 or ≥ 3 CTC at different times of Follow-Up**

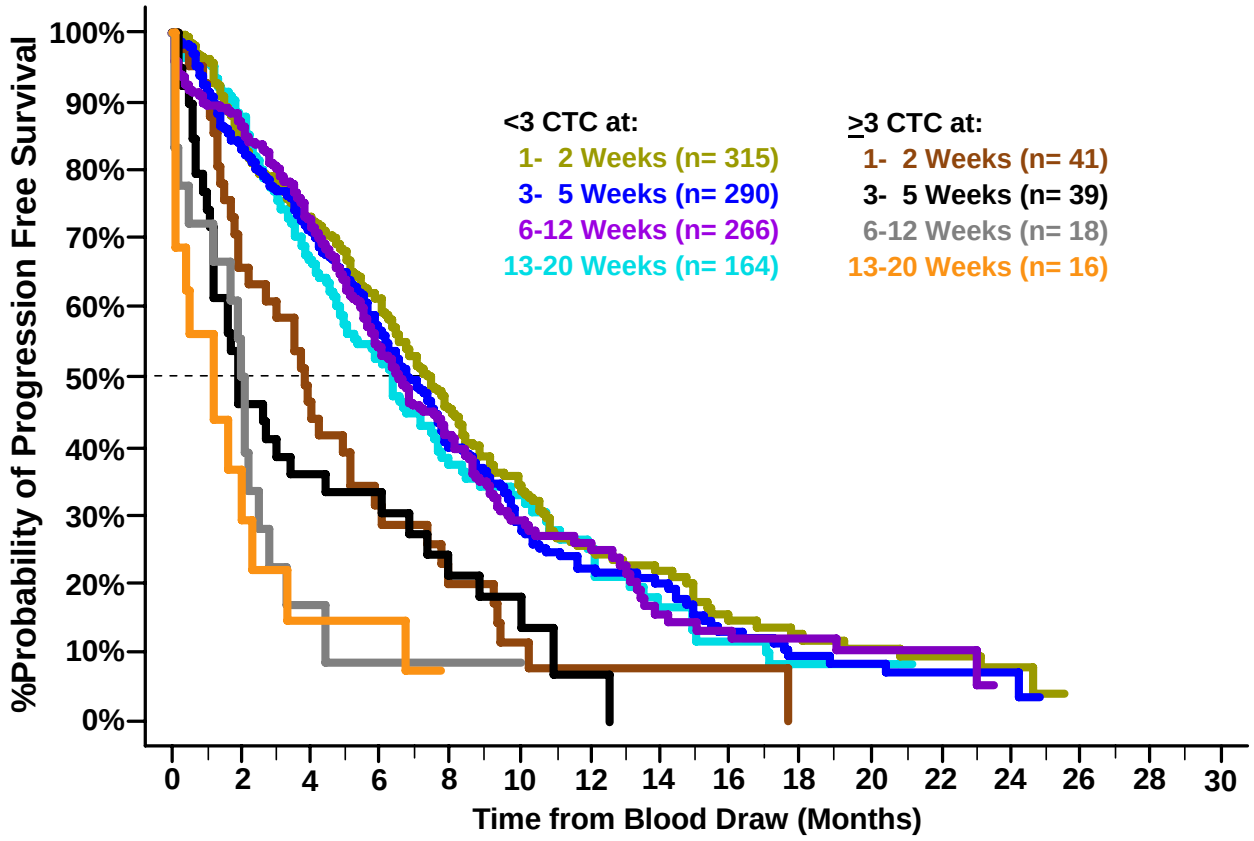



**Table 5** summarizes the results of the PFS analysis using the CTC levels and a threshold of ≥3 CTC/7.5mL at each of the different blood draw time points.

**Table 5: Progression Free Survival (PFS) for MCRC patients with <3 or ≥ 3 CTC at different time points**

| 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|
| **Sampling Time After Tx Initiation** | **N** | **≥3 CTC** | **Median PFS in Months (95% CI)** | | **Log-rank p-value** |
| | | | **<3 CTC** | **≥3 CTC** | |
| Baseline | 413 | 108 (26%) | 7.9 (7.0 - 8.6) | 4.5 (3.7 - 6.3) | 0.0002 |
| 1-2 Weeks | 356 | 41 (12%) | 7.3 (6.5 - 8.1) | 3.8 (1.9 - 5.1) | <0.0001 |
| 3-5 Weeks | 329 | 39 (12%) | 6.8 (6.1 - 7.6) | 1.9 (1.2 - 4.4) | <0.0001 |
| 6-12 Weeks | 284 | 18 (6%) | 6.5 (5.8 - 7.7) | 2.0 (0.5 - 2.5) | <0.0001 |
| 13-20 Weeks | 180 | 16 (9%) | 6.3 (4.9 - 7.4) | 1.2 (0.1 - 2.3) | <0.0001 |

As illustrated in **Figure 4** and **Table 5**, **MCRC** patients with elevated CTC (≥3

CTC/7.5mL whole blood) at any of the time points had a much higher likelihood of rapid progression than did those with <3 CTC. **Table 5** column 4 shows the median PFS times for those patients with <3 CTC ranged from 6.3 to 7.9 months and were substantially longer than the median PFS times for those patients with ≥3 CTC, which ranged from 1.2 to 4.5 months (column 5).

## Reduction or Increase in CTC Predicts Improved or Decreased PFS

Elapsed PFS times were calculated from the baseline blood draw. For Kaplan-Meier analysis (**Figure 5**), MCRC patients were segmented into four groups based upon their CTC counts at baseline, 1-2 weeks, 3-5 weeks, 6-12 weeks, and 13-20 weeks:

- Group 1 (**green** curve), 303 (70%) patients with <3 CTC at all time points. Seven (2%) of these patients only had a baseline blood draw while eight (3%) had a single blood draw between their first and last blood draw that had ≥3 CTC;

- Group 2 (**blue** curve), 74 (17%) patients with ≥3 CTC prior to the initiation of therapy but who had decreased to <3 CTC at the time of their last blood draw;

- Group 3 (**orange** curve), 29 (7%) patients with <3 CTC at an early draw (baseline, 1-2 weeks, and/or 3-5 weeks) but who increased to ≥3 CTC at the time of their last blood draw;

- Group 4 (**red** curve), 24 (6%) patients with ≥3 CTC at all time points. Three (13%) of these patients had only a baseline blood draw, one (4%) had only a 3-5 week blood draw, and one (4%) had a single blood draw between their first and last blood draw that had <3 CTC.

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

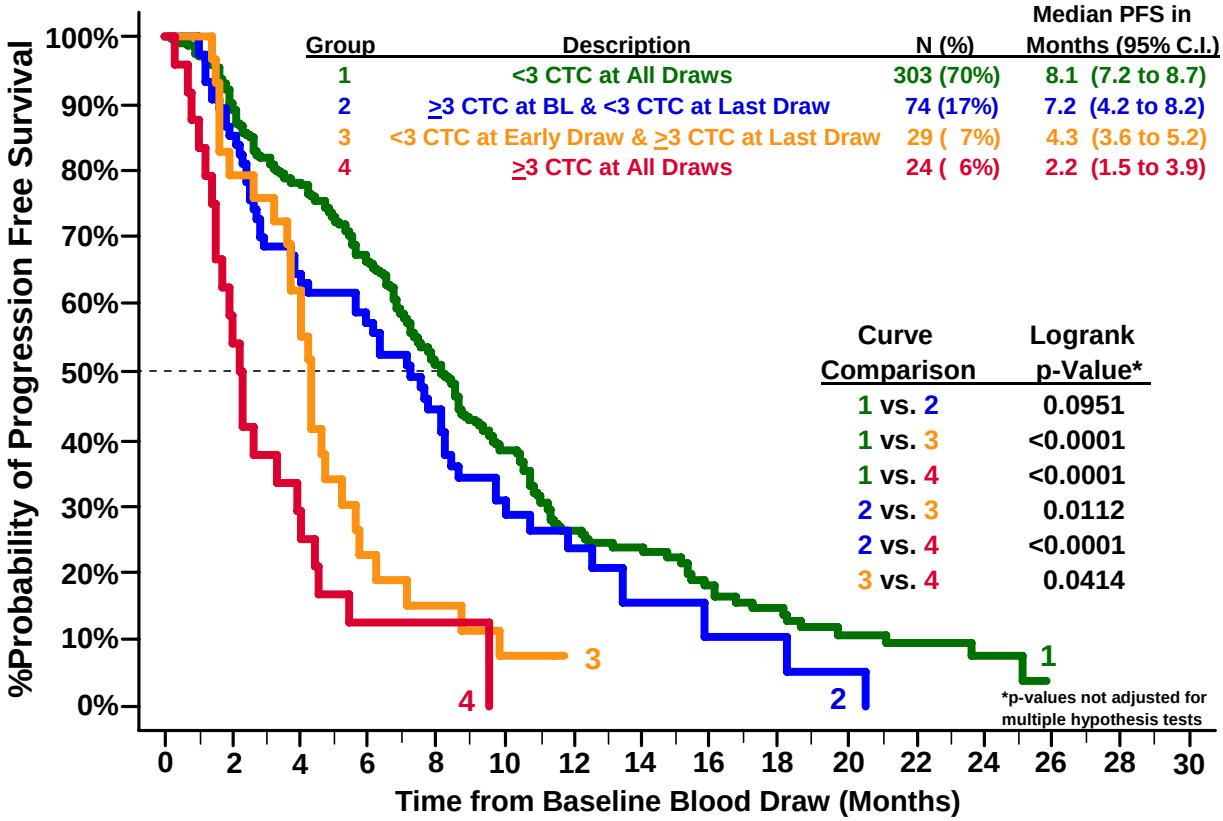



**Figure 5** shows that MCRC patients with ≥3 CTC at all time points (**Group 4**) had the shortest median PFS, which was significantly different compared to the median PFS of **Group 3**, **Group 2** and **Group 1**. The difference in the median PFS between those patients who showed a CTC reduction after the initiation of therapy (**Group 2**) was significantly longer compared to those patients who showed a CTC increase (**Group 3**).

1.3 Overall Survival (OS) Analysis of MCRC Patients

## OS Analysis Using Baseline CTC Results

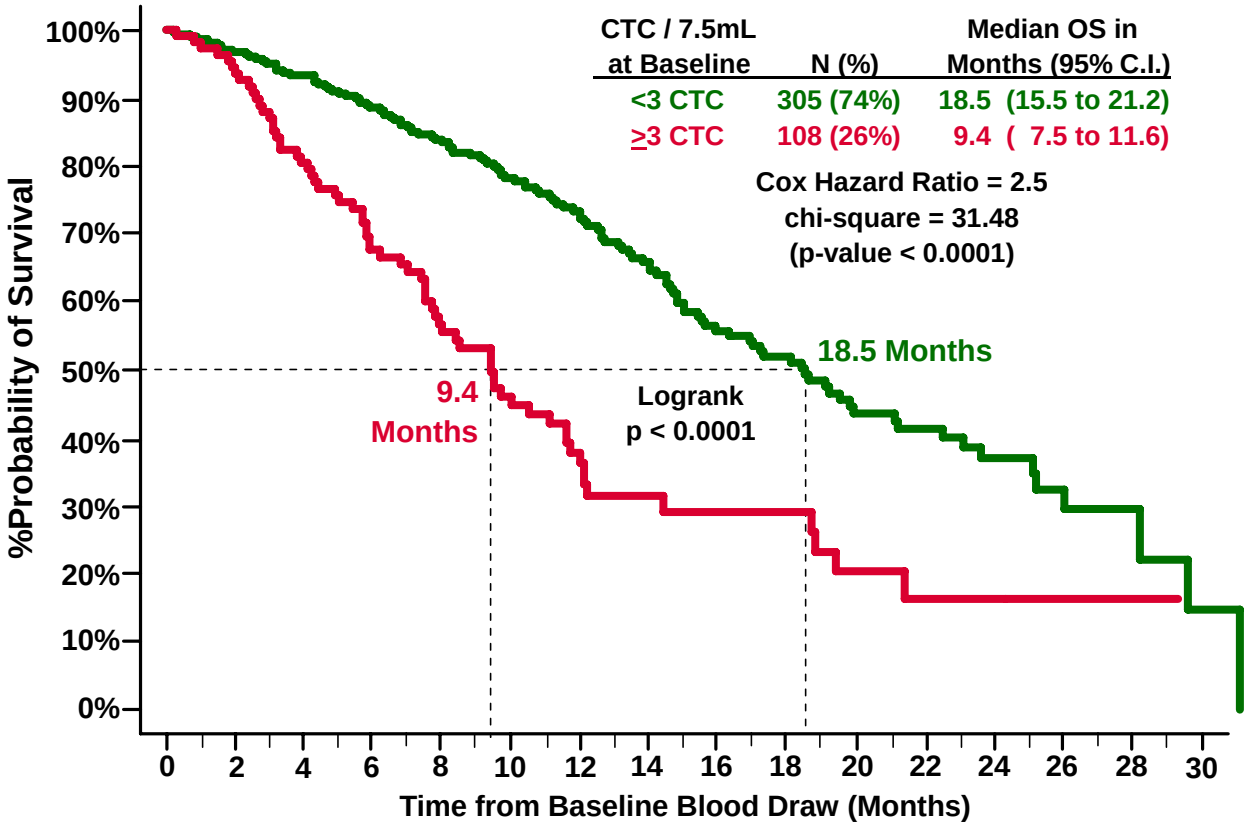
Death occurred in 202 (47%) of the 430 MCRC patients, with a mean follow-up time for the 228 (53%) patients still alive of 12.6 ± 6.5 months (median = 11.0, range = 0.8 – 30.0). At the time of these analyses, 124 (41%) of 305 patients from Favorable group (<3 CTC at baseline) compared to 68 (63%) of 108 from Unfavorable group (≥3 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=305), represented in **green**, consisted of patients with <3 CTC.
- The Unfavorable group (N=108), represented in **red**, consisted of patients with ≥3 CTC.

Median OS was significantly longer in the Favorable group compared to the Unfavorable group (18.5 vs. 9.4 months, respectively). These results are illustrated in **Figure 6**.

**Figure 6: OS of MCRC Patients with < 3 or ≥ 3 CTC at Baseline (N=413).**




## OS Using Follow-up CTC Results

The Kaplan-Meier analyses of both MCRC patient groups at each of the different follow-up blood draw times after initiation of therapy are illustrated in **Figure 7**. This figure illustrates the ability of CTC in patients with <3 and ≥3 CTC 1-2 weeks, 3-5 weeks, 6-12 weeks and 13-20 weeks after the initiation of therapy to predict time to death in 421 patients with metastatic colorectal 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 <3 CTC,
- The Unfavorable group, represented in **brown**, **black**, **grey**, and **orange**, consisted of patients with ≥3 CTC.

**Figure 7: OS of MCRC Patients with < 3 or ≥ 3 CTC at different times of Follow-Up.**

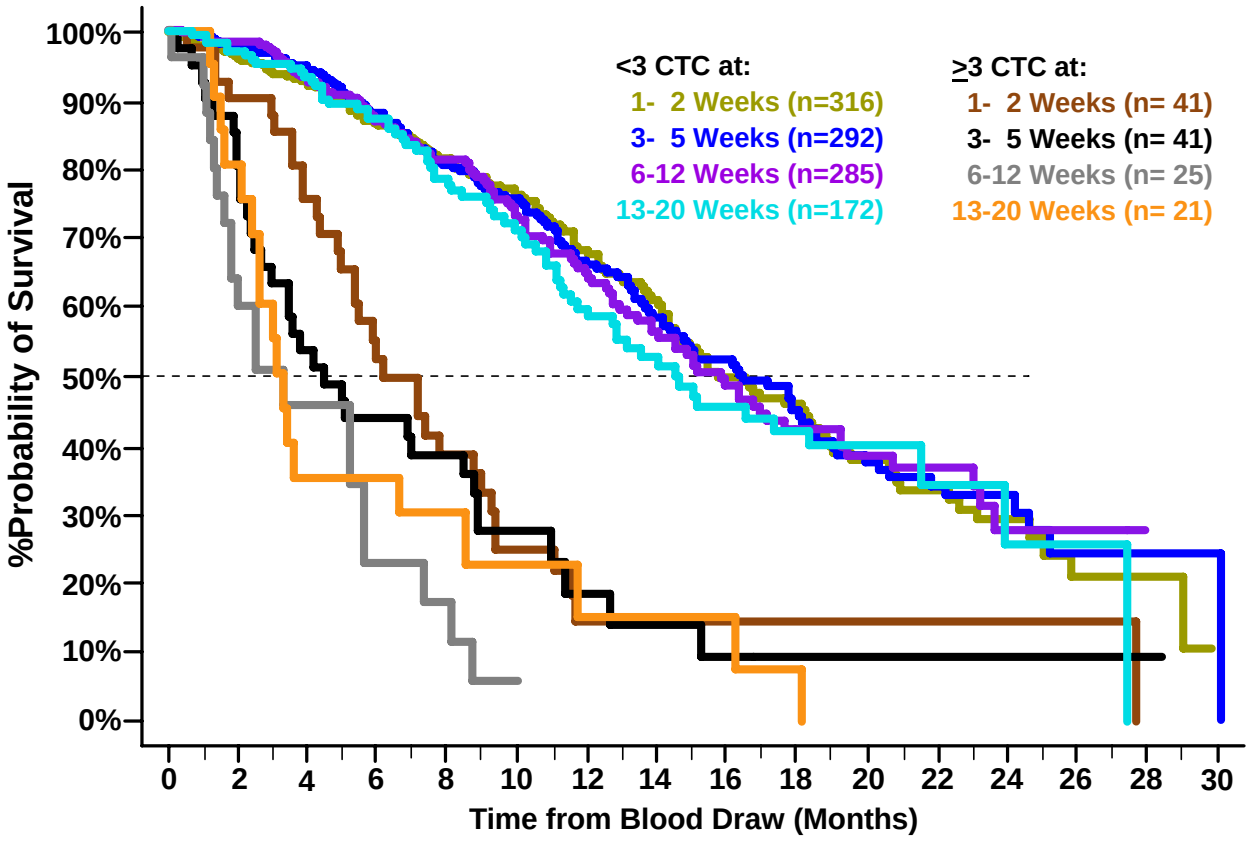



**Table 6** summarizes the results of the OS analysis using the CTC levels and a threshold of ≥3 CTC/7.5mL at each of the different blood draw time points.

**Table 6: Overall Survival (OS) for MCRC patients with <3 or ≥ 3 CTC at different time points**

| 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|
| **Sampling Time After Tx Initiation** | **N** | **≥3 CTC** | **Median OS in Months (95% CI)** | | **Log-rank p-value** |
| | | | **<3 CTC** | **≥3 CTC** | |
| Baseline | 413 | 108 (26%) | 18.5 (15.5 - 21.2) | 9.4 (7.5 - 11.6) | <0.0001 |
| 1-2 Weeks | 357 | 41 (11%) | 15.7 (14.3 - 18.4) | 6.1 (4.9 - 8.9) | <0.0001 |
| 3-5 Weeks | 333 | 41 (12%) | 16.4 (14.1 - 18.3) | 4.4 (2.6 - 8.7) | <0.0001 |
| 6-12 Weeks | 310 | 25 (8%) | 15.8 (13.8 - 19.2) | 3.3 (1.8 - 5.6) | <0.0001 |
| 13-20 Weeks | 193 | 21 (11%) | 14.6 (12.0 - 21.5) | 3.3 (2.4 - 8.5) | <0.0001 |

As illustrated in **Figure 7** and **Table 6** in columns 4 & 5, MCRC patients with ≥3 CTC at any of the time points had a much higher likelihood of dying sooner than did those with <3 CTC. The median OS times for those patients with <3 CTC ranged from 14.6 to 18.5 months and were substantially longer than the median OS times for those patients with ≥3 CTC, which ranged from 3.3 to 9.4 months.

## Reduction or Increase in CTC Predicts Improved or Decreased OS

Elapsed OS times were calculated from the baseline blood draw. For Kaplan-Meier analysis (**Figure 8**), MCRC patients were segmented into four groups based on their CTC counts at baseline, 1-2 weeks, 3-5 weeks, 6-12 weeks, and 13-20 weeks:

- Group 1 (**green** curve), 303 (70%) patients with <3 CTC at all time points. Seven (2%) of these patients only had a baseline blood draw while eight (3%) had a single blood draw between their first and last blood draw that had ≥3 CTC;

- Group 2 (**blue** curve), 74 (17%) patients with ≥3 CTC prior to the initiation of therapy but who had decreased to <3 CTC at the time of their last blood draw;

- Group 3 (**orange** curve), 29 (7%) patients with <3 CTC at an early draw (baseline, 1-2 weeks, and/or 3-5 weeks) but who increased to ≥3 CTC at the time of their last blood draw;

- Group 4 (**red** curve), 24 (6%) patients with ≥3 CTC at all draw time points. Three (13%) of these patients had only a baseline blood draw, one (4%) had only a 3-5 week blood draw, and one (4%) had a single blood draw between their first and last blood draw that had <3 CTC.

**Figure 8: A Reduction in CTC Below 3 After the Initiation of Therapy Predicts Longer OS whereas an Increase in CTC Count to 3 or above Predicts Shorter OS in MCRC Patients**

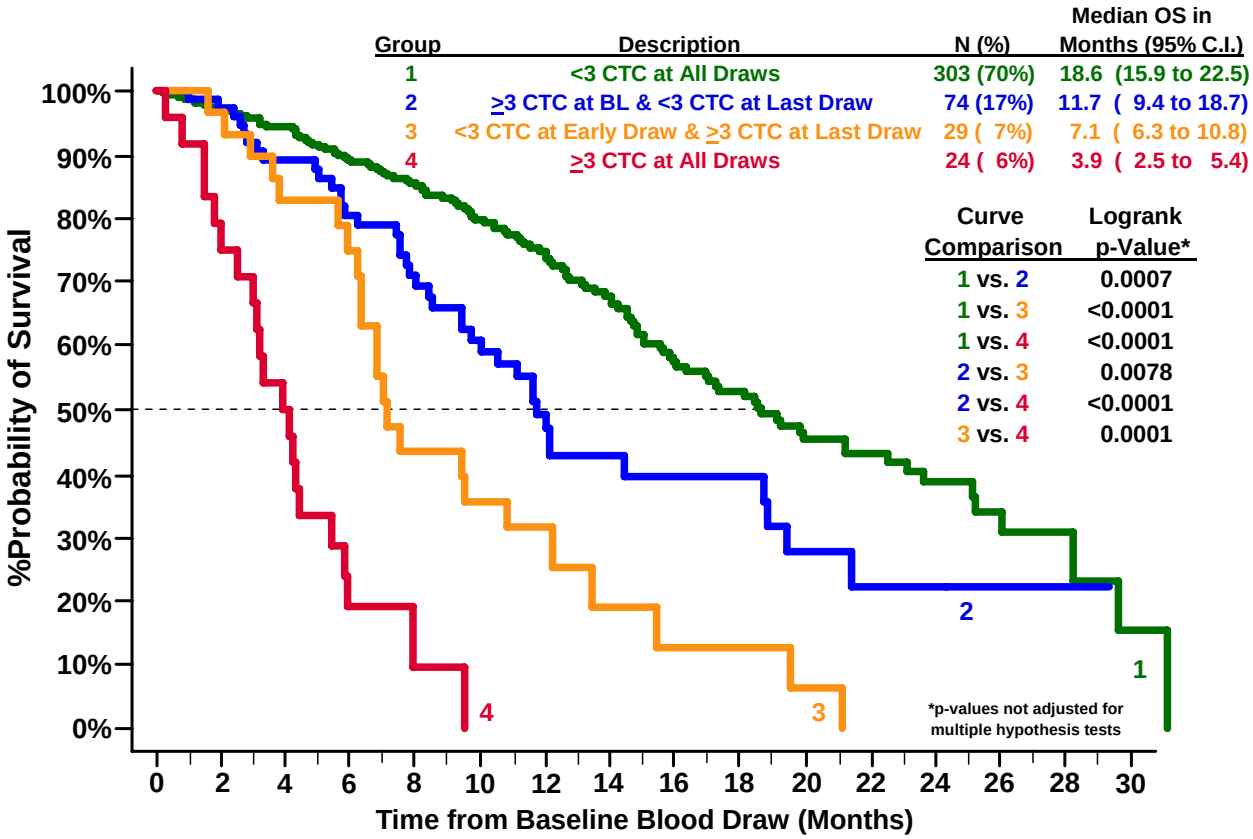



**Figure 8** shows that MCRC patients who exceed the threshold of 3 CTC at any point after the initiation of therapy had a much higher likelihood of dying sooner. Patients with ≥3 CTC at all time points (**Group 4**) had the shortest median OS, which was significantly different compared to the median OS of **Group 3**, **Group 2** and **Group 1**. Patients with <3 CTC at all time points (**Group 1**) had the longest median OS, which was significantly different compared to the median OS of **Group 4**, **Group 3** and **Group 2**. **Figure 8** also shows that patients who show a decrease in CTC (**Group 2**) have a significantly lower risk of death compared to those patients with an increase in CTC (**Group 3**).

## Univariate Cox Regression Analysis in MCRC Patients

The following parameters were analyzed using Univariate Cox regression analysis to evaluate association with PFS and OS: gender, stage of disease at diagnosis (1-4), time to metastasis (continuous), patient age (≥65 or <65), site of primary disease (colorectal or rectal or colon), ECOG status before initiation of a new line of therapy (0-2), line of therapy (1$^{nd}$ or 2$^{nd}$ or 3$^{rd}$), presence of liver metastasis (yes or no), type of therapy (bevacizumab, irinotecan, and/or oxaliplatin included or not), baseline CTC counts (≥3 or <3 CTC/7.5mL), and follow-up CTC counts 1-2 weeks, 3-5 weeks, 6-12 weeks and 13-20 weeks after the initiation of therapy (≥3 or <3 CTC/7.5mL). **Table 7** shows the results of this analysis and presents the Cox hazard ratio (HR) and associated p-value (Wald test of

Z statistic) as well as the number of patients in each evaluation.

**Table 7: Univariate Cox Regression Analysis**

| Parameter | Categories | | # of MCRC Patients | PFS Risk from Baseline | | OS Risk from Baseline | |
|---|---|---|---|---|---|---|---|
| | Positive | Negative | | HR | p-value | HR | p-value |
| **Gender** | **Male (1)** | **Female (0)** | **430** | **1.01** | **0.944** | **1.23** | **0.156** |
| **Stage at Primary Diagnosis** | **4 vs. 3 vs. 2 vs. 1** | | **407** | **0.98** | **0.734** | **1.09** | **0.330** |
| **Time to Metastasis** | **Time in Years** | | **428** | **1.00** | **0.901** | **0.92** | **0.121** |
| **Age at Baseline Blood Draw** | **≥65 Years** | **<65 Years** | **430** | **1.65** | **<0.001** | **1.82** | **<0.001** |
| **Site of Primary Disease** | **Colorectal (2) vs. Rectal (1) vs. Colon (0)** | | **429** | **1.03** | **0.733** | **1.02** | **0.866** |
| **Baseline ECOG Status** | **2 vs. 1 vs. 0** | | **414** | **1.32** | **0.002** | **1.65** | **<0.001** |
| **Line of Therapy** | **3 vs. 2 vs. 1** | | **430** | **2.04** | **<0.001** | **1.63** | **<0.001** |
| **Liver Mets** | **Yes** | **No** | **430** | **0.86** | **0.225** | **1.23** | **0.198** |
| **Bevacizumab** | **Yes** | **No** | **405** | **0.54** | **<0.001** | **0.62** | **0.001** |
| **Irinotecan** | **Yes** | **No** | **405** | **1.51** | **0.001** | **1.39** | **0.029** |
| **Oxaliplatin** | **Yes** | **No** | **405** | **0.53** | **<0.001** | **0.69** | **0.008** |
| **Baseline CTC Number** | **≥3** | **<3** | **413** | **1.59** | **<0.001** | **2.48** | **<0.001** |
| **1 - 2 Week CTC Number** | **≥3** | **<3** | **357** | **2.02** | **<0.001** | **3.23** | **<0.001** |
| **3 - 5 Week CTC Number** | **≥3** | **<3** | **334** | **2.19** | **<0.001** | **4.23** | **<0.001** |
| **6 - 12 Week CTC Number** | **≥3** | **<3** | **314** | **4.59** | **<0.001** | **10.88** | **<0.001** |
| **13 - 20 Week CTC Number** | **≥3** | **<3** | **203** | **5.07** | **<0.001** | **4.88** | **<0.001** |

## Multivariate Cox Regression Analysis in MCRC Patients

Multivariate Cox regression analyses were conducted to evaluate the independent predictive power of CTC count by adjusting for the effects of the known important clinical factors that are statistically significant in the univariate analyses. CTC were found to be strong predictors of PFS and OS (**Table 8**).

**Table 8: Multivariate Cox Regression Analysis**

| Variable | N | PFS Risk from Baseline | | OS Risk from Baseline | |
|---|---|---|---|---|---|
| | | Hazard Ratio | p-value | Hazard Ratio | p-value |
| **Baseline CTC (<3 vs. ≥3)** | 373 | 1.76 | <0.001 | 2.46 | <0.001 |
| Age at Baseline (<65 vs. ≥65) | | 1.47 | 0.002 | 1.84 | <0.001 |
| Baseline ECOG Status (0 vs. 1 vs. 2) | | 1.16 | 0.107 | 1.48 | 0.001 |
| Line of Therapy (1st vs. 2nd vs. 3rd) | | 1.59 | <0.001 | 1.41 | 0.009 |
| Bevacizumab (No vs. Yes) | | 0.65 | 0.001 | 0.68 | 0.021 |
| Irinotecan (No vs. Yes) | | 0.76 | 0.156 | 1.25 | 0.363 |

| | | | | | |
|---|---|---|---|---|---|
| Oxaliplatin (No vs. Yes) | | 0.57 | 0.002 | 1.00 | 0.984 |
| | | | | | |
| **1 - 2 Week CTC (<3 vs. ≥3)** | 321 | 1.76 | 0.003 | 2.77 | <0.001 |
| Age at Baseline (<65 vs. ≥65) | | 1.53 | 0.001 | 1.85 | <0.001 |
| Baseline ECOG Status (0 vs. 1 vs. 2) | | 1.26 | 0.025 | 1.54 | 0.001 |
| Line of Therapy (1st vs. 2nd vs. 3rd) | | 1.76 | <0.001 | 1.62 | 0.001 |
| Bevacizumab (No vs. Yes) | | 0.66 | 0.003 | 0.77 | 0.156 |
| Irinotecan (No vs. Yes) | | 0.67 | 0.066 | 1.25 | 0.402 |
| Oxaliplatin (No vs. Yes) | | 0.53 | 0.002 | 0.97 | 0.904 |
| | | | | | |
| **3 - 5 Week CTC (<3 vs. ≥3)** | 302 | 2.35 | <0.001 | 4.54 | <0.001 |
| Age at Baseline (<65 vs. ≥65) | | 1.58 | 0.001 | 2.06 | <0.001 |
| Baseline ECOG Status (0 vs. 1 vs. 2) | | 1.16 | 0.149 | 1.33 | 0.032 |
| Line of Therapy (1st vs. 2nd vs. 3rd) | | 1.74 | <0.001 | 1.65 | 0.001 |
| Bevacizumab (No vs. Yes) | | 0.68 | 0.007 | 0.86 | 0.410 |
| Irinotecan (No vs. Yes) | | 0.58 | 0.012 | 0.99 | 0.966 |
| Oxaliplatin (No vs. Yes) | | 0.47 | <0.001 | 0.88 | 0.594 |
| | | | | | |
| **6 - 12 Week CTC (<3 vs. ≥3)** | 279 | 3.04 | <0.001 | 9.43 | <0.001 |
| Age at Baseline (<65 vs. ≥65) | | 1.43 | 0.013 | 1.73 | 0.005 |
| Baseline ECOG Status (0 vs. 1 vs. 2) | | 1.30 | 0.027 | 1.53 | 0.004 |
| Line of Therapy (1st vs. 2nd vs. 3rd) | | 1.73 | <0.001 | 1.20 | 0.282 |
| Bevacizumab (No vs. Yes) | | 0.61 | 0.001 | 0.82 | 0.337 |
| Irinotecan (No vs. Yes) | | 0.78 | 0.258 | 1.47 | 0.181 |
| Oxaliplatin (No vs. Yes) | | 0.62 | 0.020 | 1.35 | 0.278 |
| | | | | | |
| **13 - 20 Week CTC (<3 vs. ≥3)** | 186 | 4.50 | <0.001 | 4.97 | <0.001 |
| Age at Baseline (<65 vs. ≥65) | | 1.26 | 0.218 | 1.55 | 0.061 |
| Baseline ECOG Status (0 vs. 1 vs. 2) | | 1.13 | 0.417 | 1.13 | 0.526 |
| Line of Therapy (1st vs. 2nd vs. 3rd) | | 1.68 | 0.004 | 1.12 | 0.628 |
| Bevacizumab (No vs. Yes) | | 0.68 | 0.058 | 0.89 | 0.655 |
| Irinotecan (No vs. Yes) | | 0.73 | 0.311 | 1.20 | 0.636 |
| Oxaliplatin (No vs. Yes) | | 0.65 | 0.135 | 1.31 | 0.477 |

1.4 Use of CTC to Monitor Clinical Status of Metastatic Colorectal Cancer

## Relationship between survival, CTC, and disease assessment by imaging

Radiological imaging is one of the primary means used to determine disease status and response to therapy in metastatic colorectal cancer patients. To establish the relationship of clinical status as determined by imaging to CTC, CTC measured at two different timepoints and imaging results were compared 1) to the true clinical endpoint overall survival and 2) to each other.

## CTC

Previous data has shown that metastatic colorectal cancer patients with ≥3 CTC / 7.5mL of blood at any succeeding follow-up visit after the initiation of therapy had a higher likelihood of progressive disease and decreased overall survival compared to patients with <3 CTC / 7.5mL of blood. The CTC results obtained 3-5 weeks after the initiation of therapy as well as the CTC results obtained within ± one month of the imaging study were classified as Favorable (<3 CTC) and Unfavorable (≥3 CTC). If more than one CTC value was obtained within ± one month of the imaging study, the CTC result obtained closest to the date of the imaging study was used.

## Imaging

Each MCRC patient had to have measurable disease, i.e. a minimum of one 2cm lesion up to and including a maximum of 10 such lesions. The method of imaging for each patient was determined by the treating oncologist in keeping with the current standard of care. Either CT or MRI of the chest, abdomen and pelvis were performed with the requirement that all lesions seen at baseline were followed using the same method for all subsequent imaging studies. Image interpretation was performed by a certified radiologist at the participating site using RECIST uni-dimensional criteria to classify each follow-up disease assessment as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD).
Each patient was imaged at a minimum of two time points up to 8 different time points. These studies included a baseline image, imaging at subsequent intervals of 2-3 months (6-12 weeks), and a final image study when the patient went off study. Copies of all patients' imaging studies were forwarded to the study coordinator at each clinical site for filing with the patient clinical data.

Out of the total of 430 evaluable MCRC patients enrolled into the study, 28 (7%) did not have a follow-up imaging study performed, 18 (4%) died before a follow-up imaging study could be performed, and 384 (89%) had one or more follow-up imaging studies performed that were assessed using RECIST criteria. At the time of the 1$^{st}$ follow-up in the 384 patients with a follow-up imaging study, 4 (1%) showed a complete response, 117 (31%) showed a partial response, 186 (48%) had stable disease, and 77 (20%) showed progressive disease. For the purposes of these analyses, patients who died before a follow-up imaging study were considered to have progressive disease.

For response to therapy at the first follow-up disease assessment, the Favorable group was defined as those having stable disease (S), partial response (PR) or a complete response (CR) by RECIST criteria (non-progressive disease, NPD) and the Unfavorable group as those with progressive disease or death (PD).

## Relationship between survival to imaging and CTC

Separate Kaplan-Meier analyses were performed to compare the overall survival of MCRC patients in the Favorable (<3 CTC) and Unfavorable (≥3 CTC) groups using CTC results at two different time points and the first follow-up imaging study. Using results from the first follow-up imaging studies performed 9.1 ± 2.9 weeks (median = 8.6 weeks) after initiation of therapy (i.e. the baseline blood draw), the median survival of the 307

(76%) patients determined by imaging to have NPD was 19.1 months (95% CI = 17.0 to 23.1) (**Figure 9, Table 9**). For the 95 (24%) patients determined by imaging to have PD, the median survival was 5.8 months (95% CI= 4.4 to 7.7).

A total of 320 MCRC patients had imaging studies performed before and after initiation of therapy or they died prior to a follow-up imaging study being performed and they had CTC assessed 3-5 weeks after initiation of therapy (average = 3.8 $\pm$ 0.7 weeks from the time of the baseline blood draw, median = 4.0 weeks). The median survival of 282 (88%) patients with Favorable CTC results (<3 CTC) was 17.3 months (95% CI = 15.0 to 19.5 months) (**Figure 10**, **Table 9**). The 38 patients (12%) with Unfavorable CTC results ($\geq$3 CTC) had a median survival of 5.4 months (95% CI = 3.6 to 9.4 months).

To determine if CTC assessments performed closer to the time of the imaging resulted in similar survival prospects compared to CTC assessments performed approximately 4 weeks after the initiation of therapy, only those patients with CTC assessments performed within $\pm$ one month of the first follow-up imaging study were analyzed (**Figure 11**, **Table 9**). Three hundred and sixty-four (364) of the 402 patients (91%) had CTC assessments within one month of the first follow-up imaging study, which was performed 9.0 $\pm$ 2.9 weeks (median = 8.5 weeks) after the initiation of therapy. The median survival of 335 (92%) patients with Favorable CTC results was 17.2 months (95% CI = 15.0 to 19.2 months). For the 29 (8%) patients with Unfavorable CTC results, the median survival was 5.4 months (95% CI = 3.2 to 7.5 months). These data show that CTC assessments at both time points provide similar results to imaging conducted approximately nine weeks after the initiation of therapy.

Applying multivariate Cox regression analysis to adjust for imaging indicates that both CTC and imaging at 6-12 weeks are independently associated with overall survival but that CTC [adjusted hazard ratio: 7.9 (4.6-13.6)] are a stronger predictor than imaging [adjusted hazard ratio: 3.1 (2.1-4.6)].

**Table 9: OS of MCRC Patients with CTC assessment approximately one month after the initiation of therapy and within one month of the radiological assessment**

| | N | Median Survival & (95% CI) in Months |
|---|---|---|
| **A. Imaging** | 402 | |
| Favorable (NPD) | 307 (76%) | 19.1 (17.0 – 23.1) |
| Unfavorable (PD) | 95 (24%) | 5.8 ( 4.4 – 7.7) |
| | | |
| **B. 3-5 week CTC** | 320 | |
| Favorable (< 3 CTC) | 282 (88%) | 17.3 (15.0 - 19.5) |
| Unfavorable (≥3 CTC) | 38 (12%) | 5.4 ( 3.6 - 9.4) |
| | | |
| **C. CTC (±1 month of Imaging)** | 364 | |
| Favorable (< 3 CTC) | 335 (92%) | 17.2 (15.0 - 19.2) |
| Unfavorable (≥3 CTC) | 29 ( 8%) | 5.4 ( 3.2 - 7.5) |

**Figure 9: Correlation of Radiological and CTC Assessment with OS: OS of MCRC Patients with NPD or PD at 1st Follow-Up Imaging Study (N=402)**

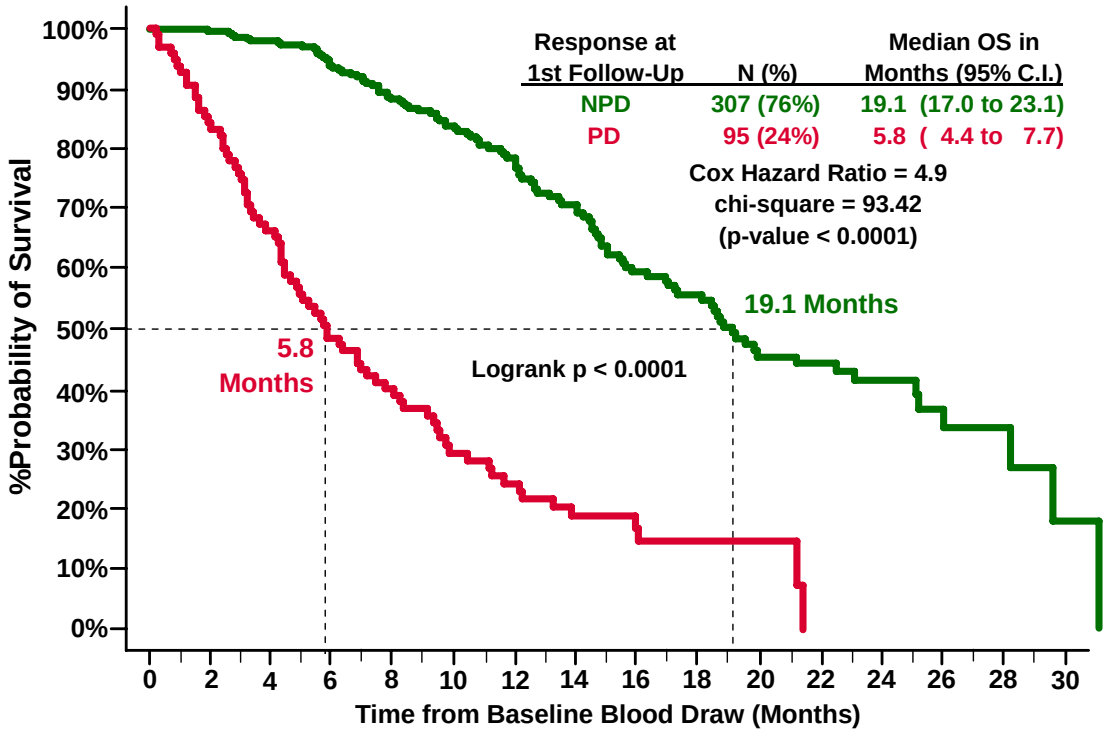


**Figure 10: Correlation of Radiological and CTC Assessment with OS: OS of MCRC Patients with <3 or ≥3 CTC at 1st Follow-Up after Initiation of Therapy (N=320)**

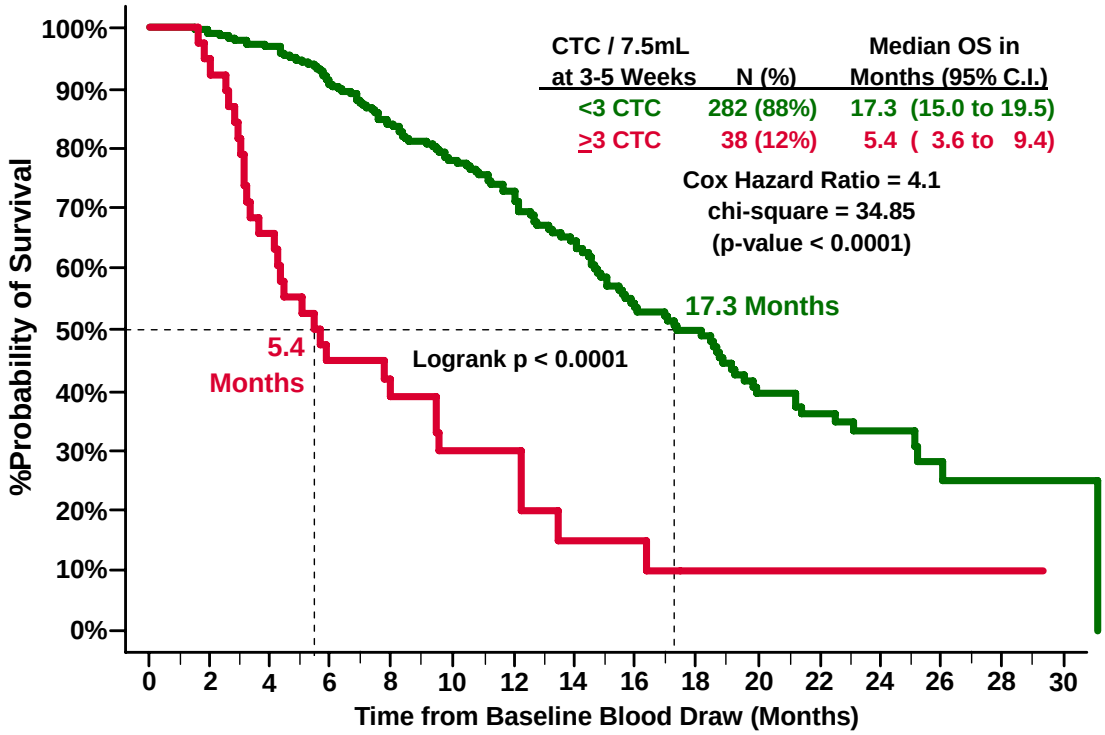
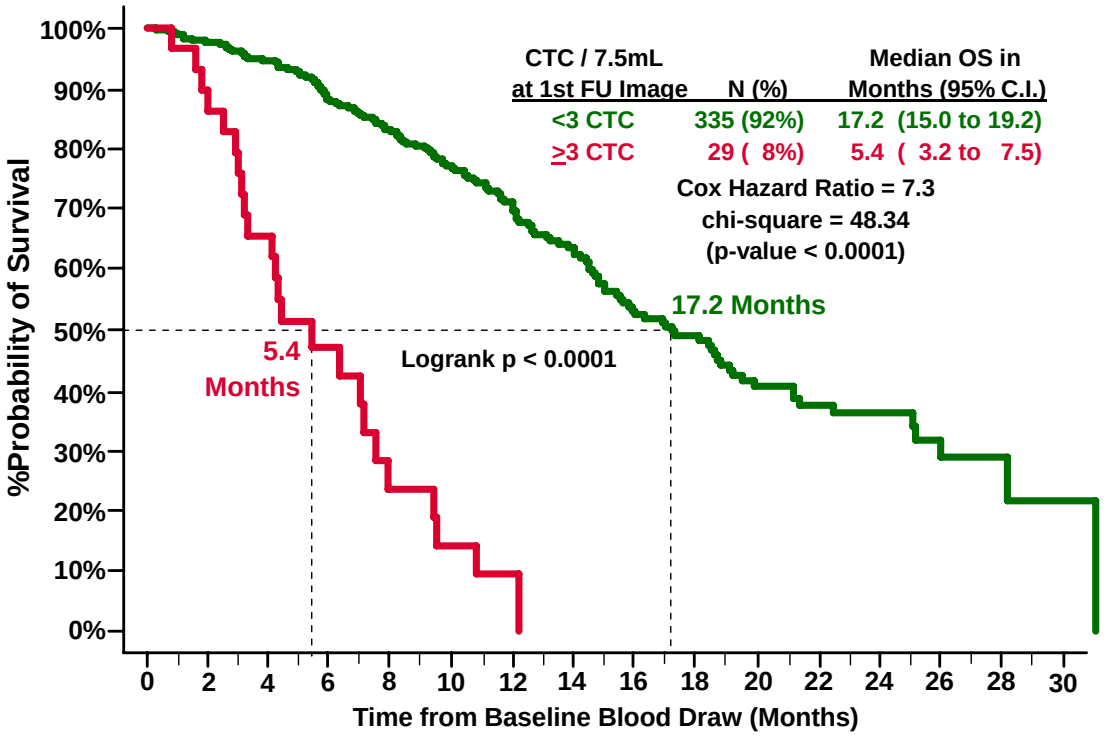


**Figure 11: Correlation of Radiological and CTC Assessment with OS: OS of MCRC Patients with <3 or ≥3 CTC within ±1 Month of 1st Follow-Up Imaging Study or Death (N=364)**




## Concordances between CTC and Radiological Monitoring in MCRC Patients

As noted above, imaging studies are a major component of the current standard of care for determining disease progression and response to treatment in the metastatic colorectal cancer setting. To further support the effectiveness of CTC in making these clinical assessments, two-by-two tabulations of concordant and discordant observations between CTC and radiological imaging were constructed.

For response to therapy, the Favorable group was defined as those having stable disease (S), partial response (PR) or a complete response (CR) by RECIST criteria (non-progressive disease, NPD) and the Unfavorable group as those with progressive disease (PD). Out of the 18 patients who died prior to a follow-up imaging study, 10 had a follow-up blood draw within 30 days of death and these 10 patients were classified as having progressive disease (PD) for the purposes of these comparisons.

The CTC results obtained within ± one month of the imaging study were classified as Favorable (<3 CTC) and Unfavorable (≥3 CTC). If more than one CTC value was obtained within ± one month of the imaging study, the CTC result obtained closest to the date of the imaging study was used. This analysis used all evaluable blood draws from the patients to match up CTC with the imaging studies, not just the ones that were selected for the designated time points as described in 1.1 above.

A total of 366 MCRC patients had CTC results within one month of the imaging study or death. The result of this "patient-wise" comparison between CTC and imaging (or death) is shown in **Table 10**.

**Table 10: MCRC Patient-Wise Comparison of CTC and Imaging**

| **Response at 1st Follow-Up Imaging Study** | **CTC within +/- 1 Month of Imaging Study or Death** | | **Total** |
|---|---|---|---|
| | <3 CTC/ 7.5mL | ≥3 CTC/ 7.5mL | |
| Non-Progressive Disease | 272 | 13 | **285** |
| Progressive Disease | 65 | 16 | **81** |
| **Total** | **337** | **29** | **366** |

| **Measurement** | **Estimate** | **Lower 95% CI** | **Upper 95% CI** |
|---|---|---|---|
| Positive % Agreement | 20% | 12% | 30% |
| Negative % Agreement | 95% | 92% | 98% |
| Positive Predictive Value | 55% | 36% | 74% |
| Negative Predictive Value | 81% | 76% | 85% |
| Overall Agreement | 79% | 74% | 83% |
| Odds Ratio | 5.2 | 2.4 | 11.2 |

Of the 384 MCRC patients with one or more follow-up imaging studies, a total of 911 imaging studies that rendered a useable radiological response were performed. A total of 805 of the 911 (88%) imaging studies had CTC results obtained within ± one month of the imaging study. Of the 18 patients who died prior to a follow-up imaging study, 10 had a follow-up blood draw within 30 days of death and these 10 patients were classified as having progressive disease (PD) for the purposes of these comparisons. The result of this "observation-wise" comparison between CTC and imaging (or death) in the 815 observations is shown in **Table 11**.

**Table 11: Observation-Wise Comparison of CTC and Imaging**

| **Response at All Follow-Up Imaging Studies** | **CTC within +/- 1 Month of Imaging Study or Death** | | **Total** |
|---|---|---|---|
| | <3 CTC / 7.5mL | ≥3 CTC / 7.5mL | |
| Non-Progressive Disease | 597 | 33 | **630** |
| Progressive Disease | 147 | 38 | **185** |
| **Total** | **744** | **71** | **815** |

| **Measurement** | **Estimate** | **Lower 95% CI** | **Upper 95% CI** |
|---|---|---|---|
| Positive % Agreement | 21% | 15% | 27% |
| Negative % Agreement | 95% | 93% | 96% |
| Positive Predictive Value | 54% | 41% | 65% |
| Negative Predictive Value | 80% | 77% | 83% |
| Overall Agreement | 78% | 75% | 81% |
| Odds Ratio | 4.7 | 2.8 | 7.7 |

In serial observations, only a minority of the transitions for imaging results between non-progressive disease and progressive disease coincided with a matching transition of CTC counts between <3 and ≥3 CTC / 7.5 mL.

Because the prognostic value of the CTC results at an earlier time-point were equivalent to that of the CTC results at the time of imaging (**Figure 10 & Figure 11**), a patient-wise comparison using results from only the 1st follow-up imaging study, performed approximately 9 weeks after the initiation of therapy, and the CTC results obtained approximately 4 weeks after initiation of therapy was constructed. A total of 320 (80%) of the 402 patients had CTC results 3-5 weeks after the initiation of therapy. The result of this "patient-wise" comparison between CTC at an earlier time point and imaging (or death) is shown in **Table 12**.

**Table 12: MCRC Patient-Wise Comparison of CTC and Imaging**

| **Response at 1st Follow-Up Imaging Study** | **CTC 3-5 Weeks After Initiation of Therapy** | | **Total** |
|---|---|---|---|
| | <3 CTC / 7.5mL | ≥3 CTC / 7.5mL | |
| Non-Progressive Disease | 228 | 18 | **246** |
| Progressive Disease | 54 | 20 | **74** |
| **Total** | **282** | **38** | **320** |

| **Measurement** | **Estimate** | **Lower 95% CI** | **Upper 95% CI** |
|---|---|---|---|
| Positive % Agreement | 27% | 17% | 39% |
| Negative % Agreement | 93% | 89% | 96% |
| Positive Predictive Value | 53% | 36% | 69% |
| Negative Predictive Value | 81% | 76% | 85% |
| Overall Agreement | 78% | 73% | 82% |
| Odds Ratio | 4.7 | 2.3 | 9.5 |

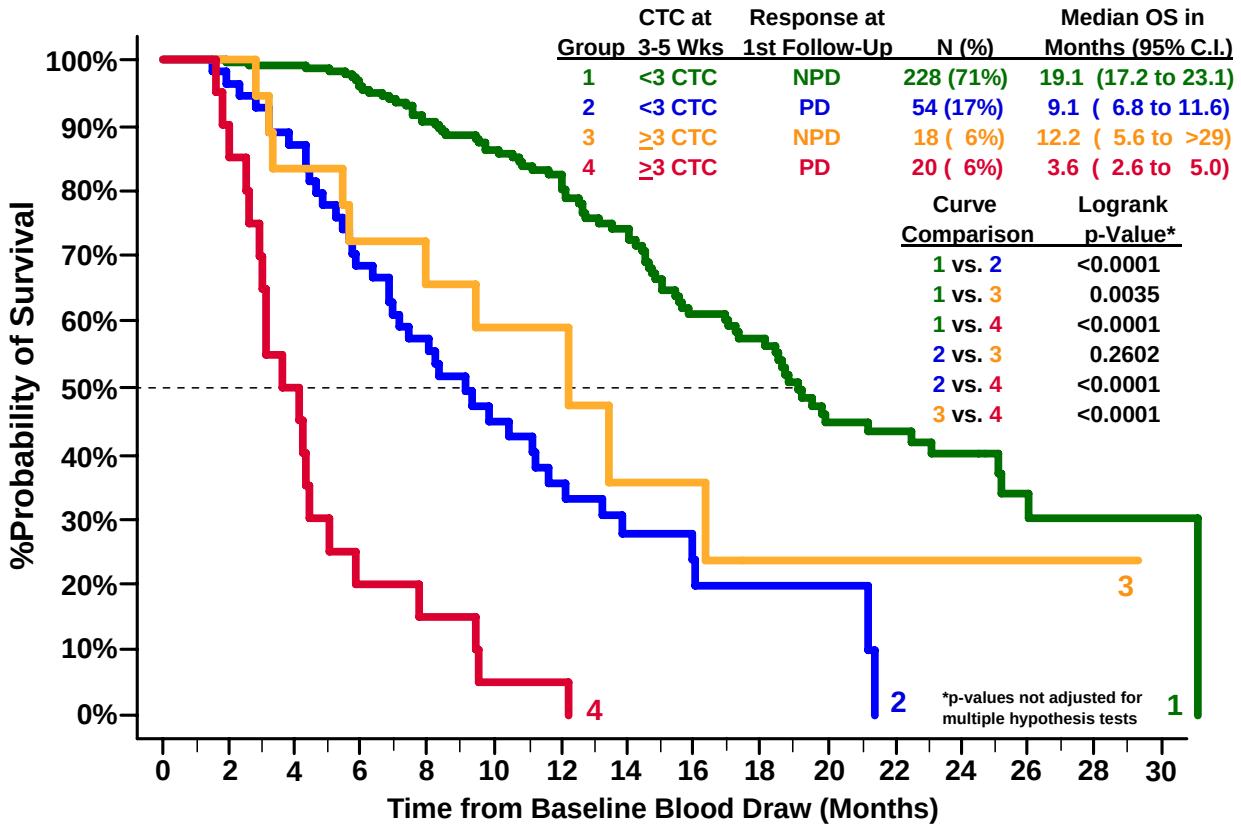
## CTC as an Adjunct to Imaging

While the overall agreement between CTC and imaging was good (approximately 78%), there was disagreement in approximately 22% of the MCRC patients. As the information from CTC assessments is intended to be used in conjunction with other diagnostic modalities to make treatment decisions, CTC assessment 3-5 weeks after the initiation of therapy and imaging in the following groups were compared to OS to determine which of the discordant results better reflected the prognosis of the patient:

- Group 1 (**green** curve), 228 (71%) patients with <3 CTC at 3-5 weeks and NPD;
- Group 2 (**blue** curve), 54 (17%) patients with <3 CTC at 3-5 weeks and PD;
- Group 3 (**orange** curve), 18 (6%) patients with ≥3 CTC at 3-5 weeks and NPD;
- Group 4 (**red** curve), 20 (6%) patients with ≥3 CTC at 3-5 weeks and PD.

**Figure 12** suggests that CTC determination is a strong independent predictor of overall survival. This figure also suggests that the combination of CTC and radiological assessments provides the most accurate assessment of prognosis.

**Figure 12: OS of MCRC Patients in Groups 1, 2, 3 and 4 using CTC 3-5 Weeks after Initiation of Therapy (n=320) and the Disease Status Determined at the 1st Follow-Up Imaging Study**




**N. Proposed Labeling:**

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

**O. Conclusion:**

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