Monitoring and Characterization of CTC in Cancer Patients

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Abstract
Concomitant with the rapidly growing number of treatment options for patients with metastatic carcinomas is the need for biomarkers to guide and monitor their use. Tumor cells shed into the blood during metastasis may help meet this need by serving as generic biomarkers for a variety of carcinomas. Indeed, assessment of circulating tumor cells (CTC) has already been shown to provide prognostic information regarding progression free and overall survival in metastatic breast (MBC), colorectal (MCRC), and prostate cancer (MPC). This has been made possible by the introduction of the CellTracks® system, which has provided a standardized method for the enumeration and characterization of CTC. In addition to predicting outcomes and monitoring treatment, CTC have also been used to detect the presence of treatment targets. The use of CTC as a source of tumor tissue can provide real-time information helpful in selecting specific therapies or families of therapies making CTC an invaluable tool in the practice of evidence-based personalized medicine.

Background and Materials
The CellSearch™ system has been used to enumerate CTC in three separate prospective multi-center registration studies involving 177 MBC, 430 MCRC and 231 MPC patients respectively. 7.5 mL of blood was drawn from patients before starting a new line of therapy and at monthly intervals after initiation of therapy. All patients in the MBC and MCRC trials had measurable disease, while all patients in the MPC study had castration resistant disease – defined as two consecutive increases in PSA despite standard hormonal management. All patients were enrolled on an Intent-to-Treat basis. Progression Free Survival and Overall Survival were measured from the time of the baseline blood draw to the diagnosis of progression, time of death or last recorded contact. Additional studies were performed to assess the presence of treatment targets on CTC.

Clinical and Translational Leads
The frequency in blood of CTC derived from the primary or metastatic tumors is extremely low. The technology developed to detect CTC is based on immunomagnetic enrichment of cells of epithelial origin from 7.5 mL blood in combination with immunofluorescent labeling followed by semi-automated microscopic identification. Assay characteristics were determined by preclinical testing. The CellTracks assay demonstrated linear recovery for 1-1000 cells from carcinoma cell lines spiked in 7.5 mL of blood. Assay specificity was determined by the absence of tumor cells in the blood of healthy donors and patients with benign diseases.

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The relation between the presence of CTC before initiation of therapy and overall survival of the MBC, MCRC and MPC patients is illustrated in Figure 1. The white bars in Panels A, B and C show the median overall survival (mOS) for patients with 0 CTC in 7.5 mL of blood, while the gray bars indicate mOS for patients with increasing numbers of CTC. The percentage of patients in each group is indicated on top of each bar. Error bars indicate the 95% confidence interval around the median survival. The median survival of patients with no CTC prior to the initiation of therapy is significantly longer compared to those patients with 1 or more CTC. At larger CTC numbers the mOS is further decreasing. While the survival prospects for patients with and without CTC are remarkably similar between the three cancers, the proportion of patients with tumor cells as well as the average number of CTC is quite different.

For MBC and MPC a threshold of 5 CTC/7.5 mL and for MCRC a threshold of 3 CTC/7.5 mL were used to stratify patients into those with Favorable outcomes (CTC <3 or <5) and those with Unfavorable outcomes (CTC ≥3 or ≥5). The Kaplan Meier curves in Figure 2 show the probability of overall survival for patients with Favorable and Unfavorable CTC counts at monthly intervals after initiation of therapy. At all time points tested the difference in survival between the Favorable and Unfavorable groups is highly significant (logrank p-values <=0.0070). Implications of these findings are that, as CTC predict outcomes at any time during treatment, they can be used to monitor treatment – any treatment. Specifically, they suggest that patients with Unfavorable CTC counts after initiation of therapy are on a futile therapy.

To answer the question as to whether a change in CTC “after” treatment has begun alters survival prospects, further Kaplan Meier analyses were performed. Patients were divided into four groups: 1) those with CTC that remained Favorable; 2) those that remained Unfavorable; 3) those that changed from favorable to Unfavorable; and 4) patients whose CTC changed from Unfavorable to Favorable during the course of therapy (Figure 3).
Fig. 2. Kaplan Meier Analysis of overall survival. Favorable CTC are indicated with 1 through 4 and Unfavorable CTC with 5 through 8.

Panel A: Median overall survival of MBC patients with Favorable CTC after 3-5 Weeks (1) (n=92), 6-8 Weeks (2) (n=77), 9-14 Weeks (3) (n=105) and 15-20 Weeks (4) (n=70) of treatment median was 21.7, 19.1, 20.8 and 20.1 months, respectively. Median overall survival of MBC patients with Unfavorable CTC after 3-5 Weeks (5) (n=40), 6-8 Weeks (6) (n=22) 9-14 Weeks (7) (n= 24) and 15-20 Weeks (8) (n=15) of treatment was 6.2, 6.3, 6.4 and 11.3 months, respectively.

Panel B: Median overall survival of MCRC patients with Favorable CTC after 1-2 Weeks (1) (n=316), 3-5 Weeks (2) (n=292), 6-12 Weeks (3) (n=285), and 13-20 Weeks (4) (n=172) of treatment was 15.7, 16.4, 15.8 and 14.6 months, respectively. Median overall survival of MCRC patients with Unfavorable CTC after 1- 2 Weeks (5) (n=41) 3- 5 Weeks (6) (n=41), 6-12 Weeks (7) (n=25), 13-20 Weeks (8) (n=21) of treatment median was 6.1, 4.4, 3.3 and 3.3 months, respectively.

Panel C: Median overall survival of MPC patients with Favorable CTC after 2-5 Weeks (1) (n=123), 6-8 Weeks (2) (n=110), 9-12 Weeks (3) (n=100) and 13-20 Weeks (4) (n=99) of treatment median was 20.7, 19.9, 19.6 and 19.8 months, respectively. Median overall survival of MPC patients with Unfavorable CTC after 2-5 Weeks (5) (n=80), 6-8 Weeks (6) (n=53), 9-12 Weeks (7) (n=49), 13-20 Weeks (8) (n=44) of treatment was 9.5, 8.5, 7.6 and 6.7 months, respectively.
Fig. 3. CTC changes after treatment in patients with MBC (Panel A), MCRC (Panel B) and MPC (Panel C). Curves labeled 1 represents patients with CTC that remain Favorable, labeled 2 CTC convert from Unfavorable to Favorable, labeled 3 CTC convert from Favorable to Unfavorable and labeled 4 CTC remain Unfavorable.

Panel A: After initiation of therapy, CTC in 83 (47%) MBC remained Favorable with median overall survival (OS) of 22.6 months, CTC in 39 (22%) patients remained Unfavorable median OS 4.1 months, CTC in 38 (21%) patients converted to Favorable CTC median OS 19.8 month and CTC converted to Unfavorable in 17 (10%) patients median OS 10.6 month.

Panel B: CTC in 303 (70%) MCRC remained Favorable median OS 18.6 months, CTC in 24 (6%) patients remained Unfavorable median OS 3.9 months, CTC in 74 (21%) patients converted to Favorable CTC median OS 11.7 month and CTC converted to Unfavorable in 29 (7%) patients median OS 7.1 month.

Panel C: CTC in 88 (38%) MPC remained Favorable median OS of more than 26 months, CTC in 71 (31%) patients remained Unfavorable median OS 6.8 months, CTC in 45 (20%) patients converted to Favorable CTC median OS 21.3 month and CTC converted to Unfavorable in 26 (11%) patients median OS 9.3 month.
all three cancers, patients with persistent CTC counts above threshold had the worst outcome. Likewise, patients that develop CTC during the course of therapy convert to a poor prognosis, similar to those with Unfavorable CTC before and after therapy. The data suggest that after the first cycle of therapy, the clinician can determine which patients are showing less than optimal response and who should therefore be placed on an alternative therapy.

As the number of therapies available to treat patients with recurrent cancer increases, oncologists are faced with the challenge of determining which therapy offers the most benefit with the fewest side effects. The shift towards targeted therapies has magnified the situation. Selecting the patient most likely to respond to a target directed drug, such as Trastuzumab (Herceptin), requires a more in-depth characterization of the patient. Unfortunately, use of archival primary tumor tissues to determine target status may not represent the disease at the time of diagnosis of recurrence. It is well-documented that due to genetic instability, a percentage of tumors continue to mutate giving rise to variants not expressing and/or resistant to a given therapeutic regimen. Consequently, assessment of therapeutic targets on CTC constitutes a “real-time” biopsy and a means of overcoming this issue.

First attempts to characterize CTC have been successful and are becoming more important in the development of targeted therapies. Figure 4 shows examples of the presence of a target protein and or its gene amplification on CTC.

![Fig. 4. Assessment of therapy targets on CTC.](image)
Panel A: Two CTC from a breast cancer patient (cytokeratin staining top panel) where one CTC expresses bcl-2 but the other CTC does not (bottom panel). Panel B: CTC from a breast cancer patient (top cytokeratin staining) expressing Her2 (bottom Her2 staining). Panel D: CTC with two copies of chromosome 17 and over-amplification of the Her2 gene. Panel C: CTC in a prostate cancer patient with over-amplification of the Androgen receptor and no translocation of TMPRSS/ERG.
Challenges and Future Directions

Measuring CTC after the first cycle of therapy has been shown to be an effective means of predicting very early on treatment efficacy. Effective therapies – ones that result in elimination of CTC – can prolong survival as shown by the improvement in overall survival in patients with MBC, MCRC and MPC that converted from an Unfavorable to Favorable CTC count. This benefit was seen in patients independent of line of therapy, indicating that treatment can still be effective in patients that have failed a previous therapy. Using this information clinically however, represents a major change in the current treatment paradigm used by most oncologists in the management of patients with metastatic breast, colorectal or prostate cancer.

Today, the current standard of care calls for routine assessments of a patient’s clinical status at or about one month intervals depending on the type of therapy. Imaging studies are an exception as they are usually performed at some intermediate timepoint and at the end of a given line of therapy. Consequently most clinicians do not entertain a change in treatment until after several cycles of drug have been administered. It is also felt that a certain minimum time, usually two to three cycles of therapy are often needed before clinical benefit may be evident. Thus the advantages associated with changing treatment at a significantly earlier timepoint must be demonstrated. To that end, clinical studies that explore whether or not an early change of therapy based on Unfavorable CTC indeed can improve survival are underway. One such study (S0500), conducted by the Southwest Oncology Group, is open and has enrolled 90 of a projected 500 patients. The trial is a prospective study using CTC counts at the end of the first cycle of chemotherapy to randomize patients with Unfavorable CTC onto a change treatment arm or continue same treatment arm. The primary objective of the trial is demonstrate a survival benefit in patients changing to another treatment after a single cycle.

Of equal importance is the use of CTC as predictive biomarkers, namely, further characterizing CTC at the gene and protein level to detect the presence of specific drug related targets. Information which can then be used to tailor treatment to meet the individual patient needs at that particular point in time. Early phase drug development studies looking at specific gene and protein markers on CTC such as HER2, c-Myc, EGFR and IGF-1R, have been successful and have led to inclusion of CTC analyses in phase I and II trials. However, there is one draw back to this application and that is a patient must of necessity have CTC present for the assessment to be made. In case few CTC are detected the technology challenge is to assure that the events detected are indeed tumor cells. Evaluation of the various errors contributing to the CTC identification pointed to the ability of the operator to assign an event as a CTC as the main contributor of the error. The presence of apoptotic CTC and CTC debris that are more frequent in patients with metastatic disease as compared to the frequency of intact CTC increases the complexity of CTC assignment. In most cases these tumor related events can not be used to assess the presence or absence of treatment targets. Alternatively one could increase the volume of blood tested the volume one can reasonably draw however quickly reaches its limit.
References


