Development of new anti-tumor drugs is time consuming and costly. Is there an effective pharmacodynamic biomarker able to predict early success or failure of a drug candidate in development? Ideally, fully validated and widely accepted biomarkers should be applied in the early stages of drug development, reducing the duration and cost of R&D activities, even as early as the pre-clinical phase. Although a relatively new procedure, early evidence indicates that a circulating tumor cell (CTC) test may fulfill this unmet need in oncology clinical trials and disease management practices.

Surviving more than a decade of developmental struggles, CTC testing is gradually becoming recognized in the clinical trial arena as an analytically validated and clinically informative assay for monitoring cancer progression. The medical literature has shown the prognostic potential of performing a CTC count in pancreatic, colon, breast, prostate, and lung cancer. As additional evidence rapidly accumulates from several large cancer centers, pharmaceutical and biotech companies are increasingly realizing the importance of the CTC test in drug development, and thus are steadily incorporating CTC assessments in clinical trials. CTC testing is exhibiting increased R&D biomarker development activity during testing of new drug candidates. This review discusses the benefits and limitations of CTC testing, focusing on application in oncology clinical trials.

CTC as a surrogate prognostic biomarker

Solid tumor cells have been known to be present in peripheral blood and bone marrow of metastatic cancer patients since 1869. Because they are such a rare event, it has taken many years for cancer researchers to develop methods to identify CTC in blood samples. One commonly used method is to enrich tumor cells by density gradient centrifugation. However, despite the basic methodology and low cost, this technique has a low recovery rate and poor separation, resulting in few tumor cells among a much higher number of normal blood cells, limiting its appeal as an effective clinical application. CTC are an ultra-rare event, making their detection extremely challenging; an estimate is one CTC in a billion blood cells.

The CellSearch™ System (Veridex LLC) is designed to enrich and enumerate CTCs in metastatic cancers. The system semi-automates and standardizes the process of CTC enrichment, cell labelling, and analytical imaging for large scale sample processing conducive to clinical trial testing. The CellSearch System is an FDA-approved in vitro diagnostic (IVD) device for managing and monitoring the progression of metastatic diseases.
including breast, colorectal, and prostate cancers. In a multi-center clinical trial, the system demonstrated that a baseline enumeration of CTC in three metastatic cancers (5 CTCs per 7.5 mL of blood sample for breast cancer and prostate cancer, and 3 CTCs per 7.5 mL of blood for colorectal cancer) differentiates between favorable and unfavorable survival outcomes. A higher CTC count correlated with a poorer outcome and overall survival, regardless of lymph node status or adjuvant therapy. Additional work from other investigators confirmed the prognostic value of CTC enumeration as a novel biomarker. As indicated in the guidelines for qualification and definition of a biomarker issued by the FDA and the NIH, CTC testing has shown several features of an effective biomarker:

**Detection.** The system defines a CTC event by positive selection, which relies on positive labelling for EpCAM and cytokeratins 8/18/19, and negative selection, which excludes hematopoietic cells positive for CD45, combined with morphologic criteria. CTCs identified and isolated by the system are visually confirmed as cancer cells.

**Prognosis.** Several independent, multicenter trials have confirmed the prognostic value of CTC detection and enumeration. Application of the CTC test can be used to track changes in enumeration over time within an individual patient in response to drug treatment; less CTC corresponded to a more favorable outcome. A recently completed trial indicated that CTC predicted which patients would benefit from treatment in prostate cancer.

**Prediction.** Enumeration of CTC, as well as molecular testing of isolated CTC to determine cancer genotype, is being actively pursued in clinical trials for determining value as predictive biomarkers for drug sensitivity, which can be used to guide treatment selection.

**Response-indicator.** CTC testing can be used to help monitor treatment effects. A trial conducted at Memorial Sloan-Kettering Cancer Center showed change of CTC from baseline strongly indicated greater risk, more than traditional markers like PSA, predicting the overall survival probability for patients with metastatic castration-resistant prostate cancer. Certain oncologists are counting CTCs every few weeks in patients with several metastatic cancers to gauge whether a treatment regimen is effective. Anti-tumor treatment is becoming increasingly more specific with molecular targeted therapy relying on defined biomarkers. The most promising outcome of CTC testing as a pharmacodynamic biomarker is clear evidence of direct pharmacological effect of a drug, which may potentially save money in drug R&D.

**Efficacy-response.** Many cancer clinics within the United States have actively adopted CTC testing as an efficacy-response tool. The Southwest Oncology Group trial was designed to test CTC as a marker for treatment efficacy in 500 metastatic breast cancer subjects. The therapy regimens are monitored and adapted as the CTC count changes.

**Treatment resistance.** CTC as a biomarker can identify subjects not responsive to treatment.

Although a relatively new test, CTC counts show promise in serving as a biomarker for testing of drug candidates and monitoring treatment response in patients with solid tumors who have progressed into metastatic stages.
CTC testing reported in clinical trials (see Table 1 – appendix)

Over the past two years, CTC testing has become a very active field in oncology drug development and pharmacodynamic studies. In an extensive literature search, prior to December 2010, only one Phase I clinical trial was published using the CellSearch™ for CTC testing as pharmacodynamic biomarker. By mid-2012, there were 18 clinical trials utilising CTC biomarker testing reported in the journal Cancer Clinical Research alone. In addition, increased R&D activities in the CTC field are evident as demonstrated by CTC lectures at international meetings and professional seminars, as well as a large number of publications available in peer-reviewed professional journals. Furthermore, at the 2012 American Society of Clinical Oncology (ASCO) meeting, a new section was exclusively devoted to the topic of CTC. Clinicians and pharmaceutical companies are using CTC count as a biomarker in many on-going clinical trials, but also use knowledge learned from CTC studies to explore new venues of personalised medicine (Table 1).

CTC testing in clinical trials (see Table 2 – appendix)

The positive rate of CTC detection in oncology patients varies and is dependent on several factors: the stage of cancer, the origin of the cancer, and the age of the patient. It has been reported that of the subjects enrolled in an oncology trial, anywhere from 10% to 78% of individuals can be positive for the presence of CTC. It is also important to determine an appropriate visit and sampling schedule for blood collection once the baseline is established, to monitor changes in CTC count. A prostate cancer trial has shown CTC testing performed at four or eight weeks after treatment can provide important prognostic information and can predict a favourable or unfavourable outcome of the therapy.

Limitations of the CTC test

Considering that the CTC test is still in a developing stage, it is worth pointing out that there are still some important issues that remain to be solved. One of most discussed weaknesses is the potential for false negative results (non-detected CTCs) by CellSearch technology or other EpCAM surface marker-based techniques. In a study which enrolled 124 metastatic prostate cancer subjects, a comparison found that a flow cytometry-based method captured more CTCs than the CellSearch System. Because of a lack of unique markers to identify tumor cells, CellSearch and other techniques relying on antibodies to capture CTC intrinsically underestimate the actual numbers. One solution being explored is to use a cocktail of cytokeratin antibodies to increase the overall detection rate. There are numerous studies exploring why tumor cells lose the expression of cell surface EpCAM. This change can be a result of epithelial-mesenchymal transition (EMT). Tumor cells undergoing EMT can be missed by the CellSearch System. Another aspect of the CTC test which can be improved is inter-operator/inter-laboratory variability. In a multicenter study, CTC results varied widely among different test sites. High inter-laboratory variability may result from inter-operator inconsistency on image interpretation, leading to misclassification of CTCs.

More options on the way

At present, CellSearch System is the only FDA-approved method as an IVD device for CTC detection and enumeration. However, several CTC isolation and capture techniques are being developed in more than two dozen academic groups and commercial ventures. Two notable methods are the CTC-chip and the ApoStream method.
The CTC-chip is also an EpCAM-based separation. It is a silicon microchip containing thousands of microposts coated with anti-EpCAM. A blood sample is passed through the microfluidic channels which contain those posts. It is a sensitive and selective detection for CTC and it also maintains the cells in an intact state so further downstream analysis for molecular profile may be performed. 17

Another new technique in this field is the ApoStream method developed by ApoCell Inc. This method relies on dielectric properties (polarizability) of cells. Because of differences in size, membrane area, density, and conductivity, CTC have distinct dielectric properties from other cells such as peripheral blood leukocytes. The ApoStream technology leverages these differences in a micro-channel flow field to isolate CTCs during a process of Dielectrophoresis Field Flow Fractionation. The benefits of this method are that it is antibody-independent and minimal cell loss occurs in the process, so it results in high recovery and high purity. Another advantage of the technique is that the isolated and recovered CTCs maintain cell viability and can be used for further downstream analysis. 20

Other techniques are being developed based on several methods: size-based isolation such as micropore filtration, imaging based methods such as EPISPOT assay, and high speed scanning fluorescence microscopy. It is possible that a CTC test in the near future will be a combination of several complementary technologies so the weaknesses of each technique may be minimised.

Summary

During testing of new oncology drug candidates, the CTC test may serve as an effective surrogate biomarker for efficacy. The CTC assay is being steadily incorporated into various phases of drug trials. The experimental design for such trials should consider the limitations of the test as well as its benefits.

References


### Trials Using CTC as a Biomarker

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Drug Candidate</th>
<th>Institutes or Country</th>
<th>Phase</th>
<th>Purpose and Results</th>
<th>Note</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Breast</strong></td>
<td>Chemotherapy</td>
<td>SWOG and NC</td>
<td>Phase III</td>
<td>Changing therapy for metastatic breast cancer (MBC) patients who have elevated circulating tumor cell levels at first follow-up assessment</td>
<td>500 patients</td>
<td>SWOG website, <a href="http://www.swog.org">www.swog.org</a></td>
</tr>
<tr>
<td></td>
<td>FEC chemotherapy</td>
<td>Germany</td>
<td>Phase III</td>
<td>Prognostic relevance of CTC</td>
<td>2,926 patients</td>
<td>Cancer Research, 70 (24 suppl 2), 2010</td>
</tr>
<tr>
<td></td>
<td>DETECT I</td>
<td>Germany</td>
<td>Phase III</td>
<td>Initially HER2- MBC and HER2+ CTC</td>
<td>1,420 MBC patients screened and 228 patients will be enrolled</td>
<td>Cancer Research, 71 (24 suppl 3), 2011</td>
</tr>
<tr>
<td></td>
<td>Clopidogrel (Plavix)</td>
<td>Germany</td>
<td>Phase II</td>
<td>Direct effect of the drug on CTC</td>
<td>48 patients enrolled</td>
<td>Cancer Research, 70 (24 suppl 2), 2010</td>
</tr>
<tr>
<td></td>
<td>LANDSCAPE study</td>
<td>France</td>
<td>Phase II</td>
<td>Measure brain metastases and CTC. Showed early CTC decrease correlated to better therapy response</td>
<td>45 patients</td>
<td>Cancer Research, 70 (24 suppl 2), 2010</td>
</tr>
<tr>
<td><strong>Prostate</strong></td>
<td>Sipuleucel-T</td>
<td>St. Bartholomew's Hospital, London, and Dendreon, Seattle, WA</td>
<td>Phase III</td>
<td>CTC as efficacy-response surrogate biomarker</td>
<td>In metastatic androgen dependent prostate cancer (mADPC): Journal of Clinical Oncology, 29, (suppl 188), 2011</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Antiandrogen therapies abiraterone acetate and MVI3100</td>
<td>Sloan Kettering Memorial Cancer Center, NY</td>
<td>Phase III</td>
<td>CTC as efficacy-response surrogate biomarker of survival, also to discover molecular determinants of CTC for predictive biomarker</td>
<td>In castration-resistant prostate cancer (CRPC): Clinical Cancer Research, 17 (12), 3903-3912 (2011)</td>
<td></td>
</tr>
<tr>
<td><strong>Lung</strong></td>
<td>Navitodax (ABT-263)</td>
<td>Johns Hopkins Cancer Center, MD</td>
<td>Phase II</td>
<td>CTC as one of end-point marker and indicate poor outcome</td>
<td>In small cell lung cancer (SCLC): Clinical Cancer Research, 18 (11), 3163-3169,3912 (2012)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pertuzumab and Erlotinib</td>
<td>Genentech, CA</td>
<td>Phase II</td>
<td>Decreased CTC count is an early indicator for longer progression-free survival</td>
<td>41 patients with non-SCLC: Clinical Cancer Research, 18 (11), 3163-3169,3912 (2012)</td>
<td></td>
</tr>
</tbody>
</table>

**Source:** Chengjun Xue, Cara G. Hill, and Thomas W. Mc Cluskey

**Table 1.** Clinicians and pharmaceutical companies are using CTC count as a biomarker in many ongoing clinical trials, but also use knowledge learned from CTC studies to explore new venues of personalized medicine.
### FAQs

<table>
<thead>
<tr>
<th>FAQ</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Why is the CTC test called a “blood biopsy”?</td>
<td>One advantage to CTC testing is the convenience of peripheral blood sampling which can provide information about a solid tumor.</td>
</tr>
<tr>
<td>How much blood volume is needed?</td>
<td>7.5 mL of whole blood is used for the test; 10 ml is drawn.</td>
</tr>
<tr>
<td>Which phlebotomy tube is used?</td>
<td>CellSave™ tube stored and transported at room temperature.</td>
</tr>
<tr>
<td>What is TAT for results?</td>
<td>It takes up to four hours to batch run samples and acquire images. Depending on how many CTC exist in a sample an experienced CTC expert can analyze and review images within hours for one CTC test.</td>
</tr>
<tr>
<td>What’s the Quality Control for the test?</td>
<td>CellSearch has QC vials with fixed, pre-labelled cancer cells.</td>
</tr>
<tr>
<td>What are the most common issues or problems with this test?</td>
<td>Blood sample quality is the major issue.</td>
</tr>
<tr>
<td>May I request other parameters in addition to enumeration?</td>
<td>Yes, however these tests are experimental. There are several molecular profiles possible, such as HER2. Another possibility is to perform FISH analysis.</td>
</tr>
</tbody>
</table>

**Source:** Chengsen Xue, Carla G. Hill, and Thomas W. McCloskey

**Table 2.** Frequently asked questions regarding the CTC test.