Circulating Tumor Cell (CTC) Enumeration Overcomes Limitations of other Blood-Based Cancer Detection Methods

The Genesis System Enables Sensitive CTC Enumeration

Liquid biopsies are rapidly gaining traction and becoming a standard of care for cancer. They are an attractive alternative to invasive, painful, costly tissue biopsies and can facilitate real-time monitoring while providing insight into the disease state, even for tissues that are difficult to access, such as brain cancers. These assays target circulating tumor cells (CTCs) or circulating tumor DNA (ctDNA).

Limitations of ctDNA as a Marker for Disease Progression

While CTCs have been known to scientists for more than a century, ctDNA has received more attention in recent years due to advances and excitement surrounding the potential for digital PCR and next-generation sequencing (NGS) technologies. However, ctDNA is found in plasma at low concentrations and NGS analysis is complicated by the extreme scarcity of some mutations and by mutations that occur with increasing age that aren’t necessarily oncogenic. Additionally, plasma can contain substantial contamination from cellular debris and genomic DNA that may not represent the current tumor state. As more sophisticated single-cell technologies have become available, there is a renewed interest in CTC enumeration and analysis.

CTC Enumeration: A Simple and Effective Predictive Biomarker

It is becoming clear the implementation of molecular and genomic characterization of CTCs can contribute to improving diagnosis and personalizing treatment selection. CTCs are cells shed by the primary tumor through the bloodstream and lymphatic systems (Figure 1). They are likely a main source of metastases, but they cannot be detected by CT or PET scans. The enumeration of CTCs has emerged as a simple and effective biomarker with many applications in cancer research for prognosis and treatment.

A unique advantage of CTCs compared to other blood-based biomarkers is they represent an entire cancer-derived cell population, providing researchers a powerful tool to study tumor heterogeneity and progression of the disease at various stages.

CTC enumeration can be used to detect early development of (micro)metastases, assess therapeutic responses of advanced disease, and select first-line treatment in various types of cancer from primary to metastatic states. It is established that detecting five or more CTCs per ml of blood in subjects with metastatic breast cancer (MBC) is associated with disease progression.

Mounting clinical evidence has prompted international experts to recommend the use of CTC enumeration for staging of metastatic breast cancer and for disease stratification in prospective clinical trials. The American Society of Clinical Oncology biomarker guidelines note that CTCs may be used in the metastatic setting to monitor these tumors during and after treatment. For example, a decrease in CTC count after one cycle of chemotherapy is indicative of tumor response, increased progression-free survival, and increased overall survival in stage III–IV cancer.
Addressing the Limitations of CTC Detection

Techniques for extracting CTCs need to be highly sensitive. For clinical adoption, analysis of CTCs must overcome a number of challenges, including their low abundance in the blood, contamination with leukocytes, loss of cell viability during purification, and tedious, low-throughput processes. Traditional techniques rely upon density gradient separation and emulsion-based technologies, which can lower the capture efficiencies even further. These techniques rely on either tumor- or epithelial-specific immune markers for the detection of the CTCs. Unfortunately, no single perfect marker can identify all CTCs, due to the inherent heterogeneity and genetic instability of cancer. The flexibility to use multiple markers or customize the markers for detection of specific CTC types enhances the accuracy and scope of capabilities for CTC enumeration. Some advantages to using the Genesis System compared to legacy systems are found in Table 1.

The Genesis System: A CTC Isolation Platform Designed for Translational and Clinical Research

Single-cell analysis is critical for unraveling cancer heterogeneity; however, researchers have very limited access to these cells for conducting downstream characterization. With many technologies, there is sample loss during the enrichment process, and the remaining contaminating white blood cells (WBCs) interfere with the results, making interpretation difficult. Very few systems have the resolution and precision required to visualize and analyze single cells.

The Genesis System supports Celselect Slides, which utilize patented microfluidics paired with 56,400 microchambers (Figure 3) to capture and isolate CTCs or other rare cells based on their size (>8 µm). WBCs captured can be quickly identified by using the WBC-specific marker CD45 and excluded from analysis.

The lack of reproducibility and sensitivity with existing technologies has limited the clinical adoption of CTCs. The Genesis System (Figure 2) was developed to analyze rare cells, including CTC isolation and enumeration. This system offers a robust solution that can accelerate clinical research and the acceptance of CTCs as a routine biomarker.

<table>
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<tr>
<th>Current CTC Detection Limitations</th>
<th>The Genesis System Advantage</th>
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<tr>
<td>Legacy systems have low capture efficiency</td>
<td>High capture efficiency, reliably detects 1 in 10⁷ cells per ml of blood</td>
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<tr>
<td>Emulsion technologies suffer WBC contamination</td>
<td>WBCs are easily excluded from analysis by immunostaining with CD45</td>
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<tr>
<td>Harsh processing methods negatively affect cell viability</td>
<td>Viable cell isolation</td>
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<td>Greater sample input required (7.5–10 ml)</td>
<td>Smaller volumes of sample (4 ml) utilized</td>
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<tr>
<td>Labor intensive with limited automation</td>
<td>Fully-automated cell capture and staining</td>
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<td>Solely dependent upon EpCAM-staining and only detect epithelial CTCs</td>
<td>Customizable staining to detect epithelial and mesenchymal CTCs for improved specificity</td>
</tr>
<tr>
<td>Designed only for enumeration of CTCs</td>
<td>Automated workflows for CTC enrichment and enumeration</td>
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Fig. 2. The Genesis System.

Fig. 3. Size-based enrichment of CTCs. A schematic of the microfluidic chambers used to capture CTCs based on their size.
1: Sample Collection ▶ 2: Isolation ▶ 3: Detection & Analysis

- Single-cell suspensions, urine, or blood samples are collected
- Cells are enriched in the Celselect Slide and isolated for downstream analysis
- Cell culture • or Microscopy • or Sequencing

Fig. 4. Cell Enumeration Workflow. Blood is directly added to the sample inlet; CTCs are isolated in the Celselect Slide while the smaller RBCs and WBCs pass through to the waste. CTCs are processed and immunostained directly in the slide. Subsequently, CTCs can be visualized and counted on the slides using an automated fluorescent imager. Contamination from larger WBCs can be excluded through image analysis.

The general workflow for CTC enumeration and enrichment is demonstrated in Figure 4. After loading whole blood onto the Genesis System, the remainder of the enrichment, purification, and staining procedures are completely automated in the Celselect Slides. If alternative means of analysis are desired, the Genesis System provides workflows to elute the collected cells for different workflows. These analysis methods can include immunohistochemistry, digital PCR, flow cytometry, fluorescence in situ hybridization (FISH) (Figure 5), sequencing (Figure 6), or even development of cell cultures. If the cells are to be counted after staining for CTC enumeration, slides are compatible with many microscopic techniques, and protocols have been validated for automatic scanning and analysis using the BioTek Lionheart, a fluorescent microscope from Agilent.

Celselect Slides enable rapid analysis without compromising cell-capture efficiency. They have been validated with several human cancer cell lines: MCF7 (breast), SKBR3 (breast), LnCAP (prostate), PC3 (prostate) and HT29 (colorectal), and capture efficiency was found to be greater than 80%. The slides captured both epithelial cancer cells, MCF7 and SKBR3, and mesenchymal cells, MDA-MB-231. Cells in clinical samples and spiked-in control samples can be collected from less than 4 ml of blood. Common samples such as peripheral blood, urine, pleural fluid, or cerebral spinal fluid samples can all be processed.

Validated Rare Cell Analysis and CTC Enumeration

A clinical study conducted at the Sidney Kimmel Cancer Center at Thomas Jefferson University revealed CTC detection in prostate cancer is more sensitive with the Genesis System than with the FDA-cleared CellSearch System (Menarini Silicon Biosystems). Analysis of 18 blood samples from patients with metastatic prostate cancer showed the Genesis System detected CTCs in 17/18 samples (94%) whereas the CellSearch System detected CTCs in only 11/18 samples (61%). CTC counts were frequently higher using the Genesis system, implying greater sensitivity for CTC detection (Figure 7).

Fig. 5. Fluorescence in situ hybridization (FISH). A, DNA FISH performed on CTCs isolated from metastatic breast cancer; B, RNA FISH performed on CTCs isolated from metastatic breast cancer.

Fig. 6. Sanger sequencing of the PCR amplicons from PC3 (A) and LNCaP (B) that were captured using Celselect Slides.

Fig. 7. Comparison of CTC counts using Celsect Slide Technology versus CellSearch Technology. Number of CTCs determined using either the Genesis System or the CellSearch System were normalized to CTC-count per 7.5 ml of blood.
The Celexselect Technology captured a very low level of leukocytes, and these cells were easily discriminated from CTCs with differential immunostaining using cell type-specific antibodies.

The cells were further analyzed and found to be prostate-specific antigen (PSA)-positive and nucleated, demonstrating they were CTCs from the prostate tumor. Not only was the Genesis System able to capture and detect more CTCs than the CellSearch System, it was able to detect subpopulations of CTCs missed by other technologies, making it a more reliable tool for monitoring.

**CTC and Expression of Cancer and Immune Markers**

In a collaborative study with IncellDx, Cytek Biosciences, and Qognit, single-cell immune and cancer marker profiling of primary tumor cells was studied to potentially predict the presence or absence of CTCs in the blood. This screening tool could be used to select patients for ongoing CTC monitoring for disease progression and response to therapy. A comprehensive study on Non-Small Cell Lung Cancer (NSCLC) was performed on patient tissue samples with paired blood.

As part of the study, the lower limits of the reproducible detection (LOD) of the Genesis System was evaluated. As few as 5 PD-L1+ CTCs were reproducibly detected in 4 ml of whole blood. (The PD-1/PD-L1 pathway is a target for NSCLC immunotherapy.) This represents a capture rate as low as 1 in 1,000,000 cells (Figure 7), and suggested a higher capture rate than the 50–60% of cells captured on legacy systems.

**CTC Enumeration as a Possible Predictor of Treatment Efficacy**

A study conducted at Juntendo University School of Medicine evaluated the use of CTC enumeration to predict eribulin treatment efficacy in MBC patients. Previous CTC enumeration systems have relied solely on EpCAM staining of CTCs to detect epithelial CTCs (eCTCs), but not all CTCs are EpCAM positive. A sub-population of CTCs with decreased levels of epithelial markers escape EpCAM-based detection. Mesenchymal CTCs (mCTCs) are not EpCAM positive. To evaluate how CTCs could be used to predict eribulin efficacy, three populations of CTCs were analyzed: eCTCs, mCTCs and total CTCs. The ability to customize stains on the Genesis System to detect mCTCs made this study possible.

![Fig. 8. CTC enumeration and analysis. A, spike in analysis for CTC recovery. NCI-441 cells (PD-L1 positive lung cancer cell line) were spiked into normal blood sample; cell recovery was reproducible down to 1 CTC in a million cells; B, representative image showing CD45 negative and PD-L1 positive cell; “y” is a variable representing the number cells collected as a function of the number of cells spiked in; “R²” is a statistical measure of fit between 0–1 that indicates how much variation of a “Cells Captured” is explained by “Cells Spiked.”](image)

![Fig. 9. Kaplan–Meier curves of PFS relative to CTC-count. The log-rank test was applied for comparisons of the survival distributions of the groups. Total CTCs including eCTCs and mCTCs was the most predictive for PFS.](image)

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Blood samples were collected from 22 patients before and during treatment. Progression-free survival (PFS) and CTCs counts were monitored by CTC type. The results demonstrate the total CTCs (including eCTCs and mCTCs) was the most predictive of PFS over eCTCs or mCTCs alone (Figure 9). Since legacy systems rely solely on detection of EpCAM positive CTCs (eCTCs), eribulin therapy monitoring with these systems would not be as accurate.

This study demonstrated that the Genesis System has the ability to customize which fluorescent stains are used, enabling the detection of multiple various CTC sub-populations and offering a significant improvement upon legacy systems.

**Summary**

The examples described in the three studies discussed above demonstrate how the Genesis System overcomes the limitations of current CTC enumeration systems as summarized in Table 1.

The Celselect technology addresses the challenges of CTC isolation and analysis by providing the following benefits:

- Capture of CTCs with high efficiency from whole blood
- Removal of 99 percent of RBCs and WBCs
- Automated workflow
- On-slide immunostaining or retrieval of viable cells for immunohistochemistry (IHC), FISH, or other types of analysis
- High-sensitivity CTC enumeration to understand tumor progression and response to therapy

The Genesis System offers an automated easy-to-use, robust, and precise approach to CTC enumeration and analysis that has the potential to implement and accelerate clinical adoption of CTCs as a biomarker in cancer diagnostics.
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References


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