Automated Circulating Tumor Cell (CTC) Isolation Overcomes Limitations of other Liquid Biopsy Methods

The Genesis System Enables Sensitive Isolation of CTCs for Enrichment or Enumeration Applications

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 non-small cell lung cancer (NSCLC). 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 researchers for more than a century, ctDNA has received more attention in recent years due to advances surrounding Next Generation Sequencing (NGS) technologies. However, ctDNA is found in plasma in low concentrations and NGS analysis is complicated by the low frequency of driver mutations. Additionally, plasma frequently contains contamination from cellular debris and genomic DNA that may not represent the current tumor state. As more sophisticated cell isolation technologies have been developed, there is renewed interest in CTCs as biomarkers.

CTC Enumeration: A Simple and Predictive Biomarker

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

A unique advantage of CTCs compared to other blood-based biomarkers is they represent a 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 the risk for early development of (micro)metastases, assess therapeutic responses of advanced disease, and select treatment for various types of cancer from primary to metastatic states. Detection of five or more CTCs per 7.5 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 metastatic breast cancer and disease stratification in prospective clinical trials. The American Society of Clinical Oncology biomarker guidelines note that CTCs may be used to monitor metastatic 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 Limitations of Legacy CTC Isolation Systems

For clinical adoption, analysis of CTCs must overcome a number of challenges, including low abundance in blood, contamination with leukocytes, and the loss of cell viability during isolation processes. Early techniques frequently relied on some type of pre-processing like density gradients or emulsion based separation. These approaches added extra processing time and were frequently accompanied by cell loss and lower capture efficiencies. In addition, many of the legacy CTC platforms rely on immune markers to detect CTCs. Unfortunately, no single perfect marker can identify all CTC types, due to the inherent heterogeneity and genetic instability of cancer. The flexibility to use multiple markers or customize markers for the detection of specific CTC types enhances the accuracy and utility of CTC enumeration.

The Genesis System: Automated CTC Isolation and Analysis Technology

The lack of reproducibility and sensitivity with existing technologies has limited the clinical adoption of CTCs. The Genesis System (Figure 2) was developed to address these shortcomings, 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.

The Genesis System supports Celselect Slides™, which features patented microfluidics paired with 56,400 microchambers (Figure 3) to capture and isolate CTCs based on their size (>8 µm). White blood cells (WBCs) captured can be quickly identified by using the WBC-specific marker CD45 and excluded from analysis. Advantages to using the Genesis System compared to legacy systems are found in Table 1.

Table 1. Advantages of the Genesis System over legacy CTC detection technologies.

<table>
<thead>
<tr>
<th>Legacy CTC Detection Limitations</th>
<th>The Genesis System Advantage</th>
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<tbody>
<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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<tr>
<td>Labor intensive with limited automation</td>
<td>Fully-automated cell capture and staining</td>
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<tr>
<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.
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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. Any liquid biopsy including peripheral blood, urine, pleural fluid, or cerebral spinal fluid can be processed on the Genesis System.

The general workflow for CTC enrichment is demonstrated in Figure 4. After loading the sample onto the Genesis System, the remainder of the enrichment, purification, and staining procedures are automated in the Celselect Slides. During enrichment, cells are retrieved in a tube for downstream analyses such as immunohistochemistry, Droplet Digital PCR, flow cytometry, fluorescence in situ hybridization (FISH) (Figure 5), sequencing (Figure 6), or development of cell cultures. If the cells are to be counted after staining for CTC enumeration (Figure 7) slides are compatible with imaging using many automated scanning microscopes.

CTC Enumeration Sensitivity

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 CellSearch System (Menarini Silicon Biosystems). Analysis of 18 blood samples from patients with metastatic prostate cancer demonstrated 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 typically higher using the Genesis System, implying greater sensitivity for CTC detection (Figure 8).
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Furthermore, this study found that Celselect Slide Technology captured low numbers 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 NSCLC primary tumor cells were examined to potentially predict the presence 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 was performed on tissue with paired blood samples to refine the prediction algorithm.

As part of the study, the lower limits of the reproducible detection (LOD) of the Genesis System were 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 greater than 1 in 1,000,000 cells (Figure 8), and suggested a higher capture rate than the cells captured on legacy systems.

CTC Enumeration as a 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 as these cells are frequently going through the Epithelial to Mesenchymal transition. 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. 9. 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; “R^2” is a statistical measure of fit between 0–1 that indicates how much variation of a “Cells Captured” is explained by “Cells Spiked.”](image)

![CTCs](image)

Fig. 10. 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.
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 10). Since legacy systems often rely on detection of EpCAM positive CTCs (eCTCs), eribulin therapy monitoring with these systems are not as accurate.

This study demonstrated that the Genesis System, with the ability to customize fluorescent stains, enables the detection of CTC sub-populations and offers a significant improvement upon legacy systems.

**CTC Enrichment Can Improve AR-V7 Sensitivity**

Researchers at the Showa University School of Medicine demonstrated that testing for the splice variant AR-V7 (the cause of castration-resistant prostate cancer) with polymerase chain reaction (PCR) can yield positive results in healthy individuals. They hypothesized that the false positivity was due to contamination of hematopoietic cells. Testing enriched CTCs were compared to whole blood. Results demonstrated that the AR-V7 false positivity rate was reduced in the CTC enriched samples. Although AR-V7 positivity did not predict therapy effectiveness, AR-V7 was more frequently positive than the Extent of Disease (EOD) and is positively correlated with progression of bone metastases suggesting AR-V7 testing on enriched CTCs is an area of exploration for prognostic testing.14

**Summary**

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

The Celselect Slide 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% of RBCs and WBCs
- Automated workflow
- On-slide immunostaining or retrieval of viable cells for downstream 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 that has the potential to accelerate the clinical adoption of CTCs as a biomarker in cancer research and potential diagnostics.
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References


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