VTX-1 Liquid Biopsy System for Fully-Automated and Label-Free Isolation of Circulating Tumor Cells with Automated Enumeration by BioView Platform

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Abstract
Clinicians continue to rely on invasive tissue biopsies as a mean to assess a patient’s disease and prescribe appropriate treatment regimens. Biopsies not only are risky and expensive but also limit the understanding of disease. Circulating tumor cells (CTCs) can be isolated from a simple blood draw and offer a promising potential to both diagnose and monitor cancer progression. The VTX-1 Liquid Biopsy System automates the isolation of clinically relevant CTC populations, while simplifying their collection for easy analysis, ultimately expanding the clinical possibilities for CTCs. We present here the key features and performance of this automated system for isolating CTCs directly from whole blood, both with cell spiking experiments and patient samples. As a first step toward the characterization of CTCs for research applications and transfer to clinical practice, we present workflows for both molecular analyses and automated cell enumeration and biomarker quantification with the BioView imaging platform.

Key terms
microfluidics; circulating tumor cells; liquid biopsy; Vortex technology

CANCER is the second leading cause of death in the US with 1,735,350 new cases expected in 2018 (https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2018.html) (1). Clinicians continue to rely on invasive tissue biopsies as a means to diagnose, assess a patient’s disease status and prescribe appropriate treatment regimens. Biopsies not only are risky and expensive but also limit the understanding of disease (2). Cancer is known to be heterogeneous, with multiple distinct tumor cell populations. Single-site tumor biopsies may not recapitulate intra-tumor heterogeneity and may fail to reflect the genetic diversity of a patient’s cancer. Furthermore, as a patient undergoes treatment, monitoring tumor evolution to allow for informed adjustments to treatment is challenging if not impossible. Circulating tumor cells (CTCs) offer a promising potential to both diagnose and monitor cancer progression (2–4). Tumor cells are known to release into the blood stream from both primary and metastatic tumors. Collecting tumor cells from circulation results in a sample representative of all tumor cell populations. These samples can be collected frequently, providing real-time monitoring of tumor evolution, treatment effectiveness and cancer metastatic risks. Complementing the sample type from tumor biopsies with CTCs may lead to earlier diagnosis and more personalized treatment of cancer ultimately resulting in a better standard of care for cancer patients.

CTCs are extremely rare cancer cells shed from primary tumor or metastases into the peripheral blood of patients with solid tumors. CTC counts have been clinically correlated with the prognosis of patients with breast, colon and prostate cancers, and their molecular characterization could provide additional insights into their functional roles in cancer progression, thereby helping to guide treatment toward personalized therapies (5–7). Isolating rare CTCs among a background of
millions of white blood cells (WBCs) and billions of red blood cells (RBCs) while preserving their utility for downstream characterization until now has been a technical challenge limiting their broader use in research and clinical laboratories.

The CellSearch system was the first instrument available for CTC isolation and remains the only CTC capture platform approved by the FDA for CTC enumeration utilized for prognosis. The CellSearch test relies on positive selection using epithelial cell surface biomarkers such as epithelial cell adhesion molecule (EpCAM) to immunomagnetically capture CTCs (6,8). This approach requires that CTCs express the markers of interest to be captured and consequently would miss the cells lacking these markers (9). More recent technologies such as Fluxion (10) or Adnagen (11), RareCyte (12), EPIC (13), and specific research workflows with CellSearch (14,15) among others (16), allow for the isolation and analysis of CTCs beyond their enumeration but still require preexisting knowledge of the molecular biomarkers expressed or require an upstream sample preparation/downstream cell picking that may lead to cell loss and/or impact cell integrity for downstream assays. To address these limitations, alternative label-free isolation of clinically relevant CTCs followed by their further analysis challenging, often resulting in CTC loss and complicating, sometimes rapid and needing minimal equipment but can make releasing the CTCs into suspension for automated blood processing. The Vortex Cartridge contains the Vortex microfluidic chip that isolates CTCs based on their physical properties. The user enters simple sample information and initiates the processing. In 1–2 h, CTCs are isolated and released into a container for downstream analysis.

VTX-1 Liquid Biopsy System for a Fully Automated Blood Processing

The VTX-1 Liquid Biopsy System was developed to fully automate the isolation of CTCs directly from whole blood samples (30,31). As described in Figure 1, the user connects a patient blood tube to the one-time-use Vortex Cartridge before inserting the cartridge into the VTX-1 System for automated blood processing. The Vortex Cartridge contains the Vortex microfluidic chip that isolates CTCs based on their physical properties. The user enters simple sample information and initiates the processing. In 1–2 h, CTCs are isolated and released into a container for downstream analysis.

CTC Capture is Unbiased by Antibody Expression

The VTX-1 system uses the physical properties of the CTCs to isolate the cells. CTCs have a different shape and deformability and are larger than white and red blood cells, resulting in their stable capture in the micro-vortices created in the microfluidic chip. Isolated CTCs are thus unbiased by molecular characteristics, enabling the isolation of the diverse population of CTCs in the patient whether they be epithelial or mesenchymal (29).

High CTC Recovery and Purity from Metastatic Cancer Patients

The VTX-1 CTC isolation process provides a high yield of CTCs with minimal white blood cell contamination. In preliminary clinical studies using the Vortex technology with an early-stage platform, CTCs were isolated with high purity (from 1.4 to 92.5 WBCs/ml blood) from patients with metastatic breast (median 40.7 CTCs per 7.5 ml; n = 22), colorectal (median 12.2 CTCs per 7.5 ml, n = 41), non-small-cell lung (NSCLC) (median 26.2 CTCs per 7.5 ml, n = 15), and prostate (median 5.6 CTCs per 7.5 ml, n = 20) cancers (Fig. 2B) (24,26,29), using a CTC identification criteria previously defined (23). A higher purity CTC sample increases the accuracy and sensitivity of downstream assays, such as cytology, next-generation sequencing, and Sanger sequencing. For example, KRAS, BRAF, and PIK3CA mutations were detected by Sanger sequencing, revealing concordance between CTCs and liver metastasis for 7/9 colorectal cancer patients (26).

Cells Collected are Preserved

Owing to the passive in-flow processing of the blood through the chip and the absence of physical restrictions or microscale filters, cells isolated with the VTX-1 system are unaltered by labels or reagents. It has been demonstrated that Vortex processing does not affect cell viability (23), impair colony and tumor spheroid formation, or alter cell invasiveness (30). Since the VTX-1 processing leaves cells viable, the collected cells are ideal for cell culture experiments, mouse CDX models, live cell assays, and any assays where it is critical that the mRNA or protein expression remains unaltered as much as possible.
Flexibility in the CTC Release for Integration with Downstream Assays

Upon isolation with the VTX-1, CTCs are released free-floating and can be collected in a container of the user’s choice. This can be an Eppendorf tube, a chamber slide, a Petri dish, or a well-strip depending on the requirements for the downstream assay. For example, for molecular assays, the CTCs would be collected into an Eppendorf tube for RNA or DNA extraction, whereas for cell culture work, the CTCs would be collected directly into a Petri dish (Fig. 1). No transfer of the sample is required, limiting manual intervention and cell loss.

Different VTX-1 Protocols for Optimal CTC Recovery or Optimal CTC Purity

The flexibility of the VTX-1 allowed us to target either optimal CTC recovery (“High Recovery Mode”) or optimal CTC purity (“High Purity Mode”, Fig. 2) depending on the intended downstream analysis. The system offers the possibility to automatically reprocess a sample: after being processed once (first cycle), the effluent is collected on the Vortex Cartridge and automatically reinjected into the Vortex chip for a second time (second cycle) or a third time (third cycle), in order to capture additional cancer cells missed in the first pass (“High Recovery Mode”, Fig. 2). When processing 50 MCF7 breast cancer cells spiked in 4 ml of blood with the high purity mode (1 cycle), 53.8% of MCF7 cells were collected on average, with only 101 contaminating WBCs/ml blood processed. When the same sample was processed with a high recovery mode (3 cycles), 71.6% of the cells were recovered, with 350 contaminating WBCs/ml (30).

CTC Characterization, from the Bench to the Bedside

Isolation of CTCs is the first step toward the characterization of these cells to both support cancer research and guide clinical decisions over the course of a patient’s disease.
Compatibility with Molecular Analyses

The VTX-1 system makes the molecular analysis of CTCs simpler by first isolating the CTCs with high purity and then presenting them in a format where DNA and RNA extraction can be easily accomplished, as needed for the assay selected. Workflows for Sanger sequencing (26), qPCR, or next-generation sequencing (27) have been previously published or presented. Furthermore, the plasma can be collected prior to isolation of the CTCs for isolation of ctDNA. We have demonstrated workflows that allow for the detection of EGFR mutations from both the ctDNA and CTCs from a single blood tube. This workflow can be applied to other gene panels or even mRNA detection in CTCs and cfRNA in the exosomes in plasma.

An Integrated Workflow with VTX-1 and BioView for fully Automated CTC Enumeration and PDL1 Quantification

For imaging-based workflows, CTCs need to be adhered onto a glass slide to enable immunostaining, cytology or FISH workflows followed by image analysis. Furthermore, manually identifying CTCs can be cumbersome and user-variable that

Figure 2. CTC recovery using the Vortex technology. (A) Validation of VTX-1 cell recovery in different modes with MCF7 cancer cell spiking experiments. Two VTX-1 modes of operation are available to favor either cancer cell purity (high purity mode, 1 cycle) or cancer cell recovery (high recovery mode, 3 cycles) (30). (B) Vortex technology was successfully applied for the CTC isolation from blood samples of patients with metastatic breast, lung, prostate and colon cancer. Each dot represents the CTC number per 7.5 ml of blood for one patient blood draw. For each cancer type, the blue bar indicates the healthy threshold (or cutoff) obtained in each cancer specific study, while the % below each category indicates the number of patients whose CTC number is above this threshold, that is, 91, 73, 80, and 80% of the patients. [Color figure can be viewed at wileyonlinelibrary.com]

Figure 3. (A) Combined workflow for fully automated blood processing and CTC isolation on a glass slide using the VTX-1 Liquid Biopsy System, immunofluorescent staining, fully automated imaging, CTC enumeration, and biomarker expression quantification using the BioView System. (B) Best in class optics and unique imaging algorithms specifically tailored to facilitate rapid 3D capture, detection, and analysis of fluorescently labeled cells within the sample, with in this example CK (green) and CD45 (red). CTCs automatically detected are presented for operator confirmation and report. (C) Example of a CTC detected during automated scan. The sample was removed from the scanning system, re-hybridized with FISH probes, and reloaded to the system. The system automatically relocates, captures, and analyzes the FISH signal pattern of selected CTCs. Left panel: CTC identification by immunofluorescence (PDL1 in yellow). Right panel: FISH analysis of ALK Break Apart probe in the preselected PDL1 positive cell. [Color figure can be viewed at wileyonlinelibrary.com]
can reduce the utility of the CTCs isolated. Vortex Biosciences collaborated with BioView to develop an integrated workflow for CTC enumeration and biomarker analysis. As seen in Figure 3, CTCs isolated with the VTX-1 can be collected and immobilized onto a microscope glass slide for downstream cytology, FISH and immunophenotyping analysis followed by automated detection and characterization of CTCs using the BioView platform. The BioView system automates image collection and then applies an algorithm for identifying CTCs based on several factors including cell size, cell shape, nucleus-to-cyttoplasm ratios, and the presence of unique biomarkers identified by target antibodies. Identified CTCs are then placed in a gallery for review by a cancer researcher or pathologist. Once cells are identified, the BioView system will register their location allowing for the fluorescent signals to be bleached. Further analysis such as identifying drug-specific protein biomarkers and DNA rearrangements by FISH can then be conducted on the registered CTCs. This integrated workflow makes both CTC counts and image analysis of CTCs simple, opening new areas of cancer research and new opportunities for clinical impact.

CONCLUSION

CTCs offer tremendous potential to transform the standard of care for cancer patients. The innovative VTX-1 Liquid Biopsy System isolates relevant CTCs with a simple, fully automated workflow. The integration with downstream analytics opens new doors in how CTCs can be used in cancer research and the clinic.

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CONFLICTS OF INTEREST

ES-C, CR, and SCC have financial interests in Vortex Biosciences and intellectual property described herein. TK and EK have financial interests in BioView and intellectual property described herein.

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