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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. © 2018 The Authors. Cytometry Part A published by Wiley Periodicals, Inc. on behalf of International Society for Advancement of Cytometry.

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 CTC enrichment technologies have been developed, such as ClearCell® FX, using the larger size of CTCs compared to RBCs and WBCs (17,18). Limitations of this method, however, include a requirement for lysing the RBCs prior to CTC isolation. Microfilter technologies, such as ScreenCell® (19), ISET® (20), CellSieveTM (21), and Parsortix (22), involve flowing blood through pores or microfluidic steps of calibrated size to trap larger CTCs while smaller blood cells pass through. Such technologies or approaches have the advantages of being less complicated, sometimes rapid and needing minimal equipment but can make releasing the CTCs into suspension for further analysis challenging, often resulting in CTC loss and higher numbers of contaminating WBCs.

Thus, there is still an unmet need for a fast, simple, and label-free isolation of clinically relevant CTCs followed by their release in suspension, in a flexible manner compatible with various downstream assays. Having a technology that both automates CTC isolation and simplifies their collection downstream should open up the clinical possibilities for CTCs. The VTX-1 Liquid Biopsy System was developed to meet these needs.

Vortex Technology for Label-Free CTC Isolation

The Vortex technology exploits inertial microfluidics and uses laminar microscale vortices to isolate and concentrate CTCs from blood. Here, CTC capture is based on cell size, cell shape, and deformability and is the agnostic of tumor biomarkers (Fig. 1A). The Vortex chip has been characterized and validated for CTC isolation from blood samples of metastatic cancer patients with breast, colon, lung and prostate cancer (23–29). Numerous workflows have been optimized for various CTC research applications, such as immunofluorescence staining and CTC enumeration (23–26,29), cytopathology and cytogenetics (24), Sanger sequencing (26), next-generation sequencing (27,29), and even Western-blotting on single cells (28) (Fig. 1).

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.

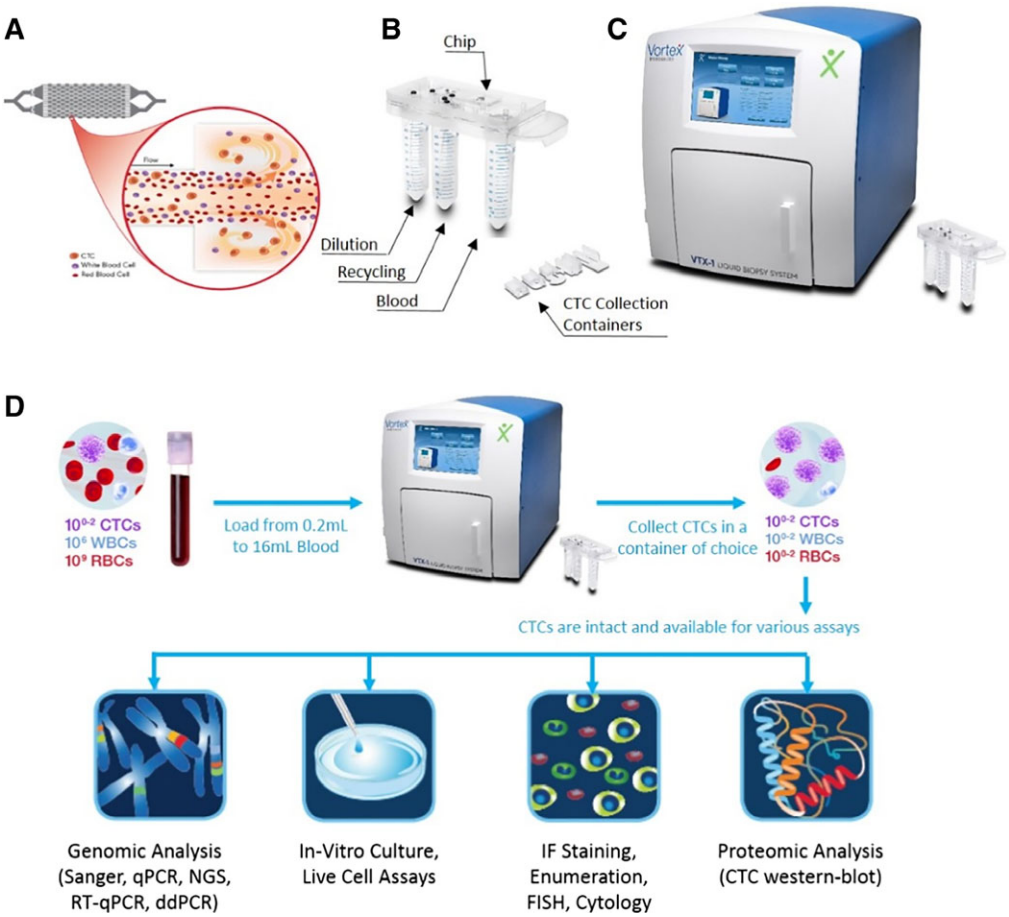

Figure 1. (**A**) The Vortex plastic chip consists of 16 parallel channels and nine serial reservoirs in each channel (30). At high flow rates, laminar microscale vortices develop in the rectangular reservoirs and trap larger cancer cells while smaller blood cells pass through (31). (**B**) The one-time use Vortex Cartridge, containing the chip, the collection container, and the blood collection tube. (**C**) Once the Vortex Cartridge is inserted into the VTX-1 Liquid Biopsy System, the blood is transferred to the dilution tube for dilution with PBS buffer and injected through the microfluidic chip for CTC capture, while the flow-through is collected into the recycling tube. After processing of the entire volume of blood and a wash step to remove any contaminating blood cells, the flow rate is lowered and vortices dissipate to release the captured cells off-chip into their collection container. Depending on the operation mode selected by the user, the blood is transferred from the recycling tube and re-injected through the chip for a second cycle. (**D**) Workflow for fully automated CTC enrichment, directly from a blood tube to the collection off-chip in various containers of the user's choice for different downstream assays. [Color figure can be viewed at wileyonlinelibrary.com]

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.

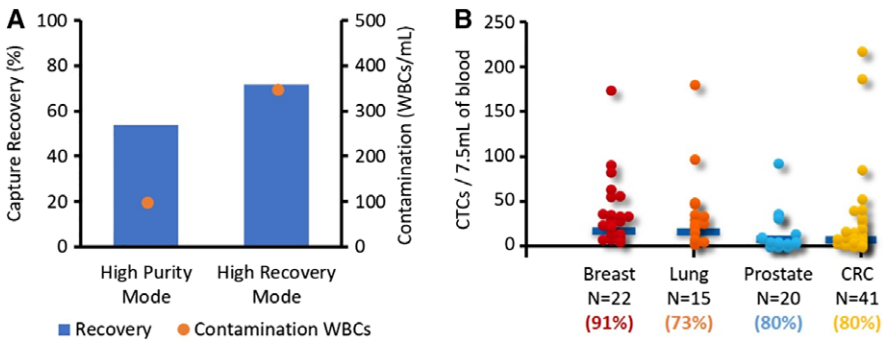

Figure 2. CTC recovery using the Vortex technology. (**A**) Validation of VTX-1 cell recovery in different modes with MCF7 cancer cell spiking experiments. Two VTX-1 modes of operation are available to favor either cancer cell purity (high purity mode, 1 cycle) or cancer cell recovery (high recovery 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]

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 the 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

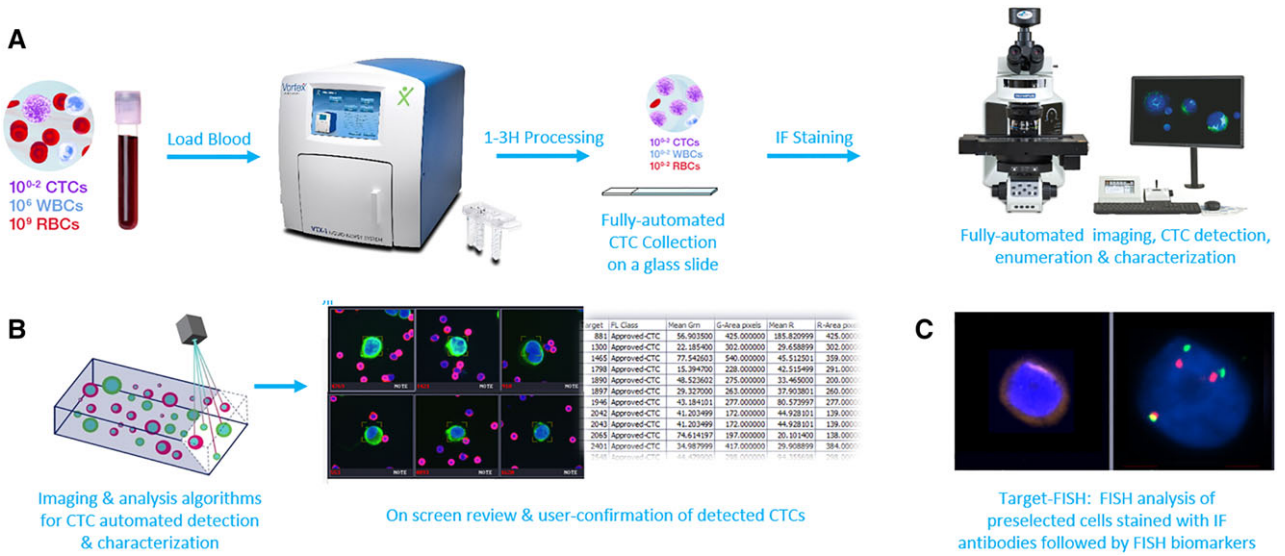

Figure 3. (**A**) Combined workflow for fully automated blood processing and CTC isolation on a glass slide using the VTX-1 Liquid Biopsy System, immunofluorescent staining, fully automated imaging, CTC enumeration, and biomarker expression quantification using the BioView System. (**B**) Best in class 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-cytoplasm 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.

Literature Cited

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA Cancer J Clin 2017; 67(1):7–30.
2. Ilie M, Hofman P. Pros: Can tissue biopsy be replaced by liquid biopsy? Transl Lung Cancer Res 2016;5(4):420–423.
3. Ignatiadis M, Lee M, Jeffrey SS. Circulating tumor cells and circulating tumor DNA: Challenges and opportunities on the path to clinical utility. Clin Cancer Res 2015; 21(21):4786–4800.
4. Alix-Panabières C, Pantel K. Clinical applications of circulating tumor cells and circulating tumor DNA as liquid biopsy. Cancer Discov 2016;6(5):479–491.
5. Danila DC, Heller G, Gignac GA, Gonzalez-Espinoza R, Anand A, Tanaka E, Lilja H, Schwartz L, Larson S, Fleisher M, et al. Circulating tumor cell number and prognosis in progressive castration-resistant prostate cancer. Clin Cancer Res 2007;13:7053–7058.
6. Danila DC, Anand A, Sung CC, Heller G, Leversha MA, Cao L, Lilja H, Molina A, Sawyers CL, Fleisher M, et al. TMPRSS2-ERG status in circulating tumor cells as a predictive biomarker of sensitivity in castration-resistant prostate cancer patients treated with abiraterone acetate. Eur Urol 2011;60:897–904.
7. Scher HI, Lu D, Schreiber NA, Louw J, Graf RP, Vargas HA, Johnson A, Jendrisak A, Bambury R, Danila D, et al. Association of AR-V7 on Circulating Tumor Cells as a treatment specific biomarker with outcomes and survival in castration resistant prostate cancer. JAMA Oncol 2016;2(11):1441–1449.
8. Racila E, Euhus D, Weiss AJ, Rao C, McConnell J, Terstappen LWMM, Uhr JW. Detection and characterization of carcinoma cells in the blood. Proc Natl Acad Sci USA 1998;95(8):4589–4594.
9. Ozkumur E, Shah AM, Ciciliano JC, Emmink BL, Miyamoto DT, Brachtel E, Yu M, Chen P-I, Morgan B, Trautwein J, et al. Inertial focusing for tumor antigen-dependent and -independent sorting of rare circulating tumor cells. Sci Transl Med 2013;5:179ra147.
10. Ramirez P, Saenz L, Cascales-Campos PA, González Sánchez MR, Llácer-Millán E, Sánchez-Lorencio MI, Díaz-Rubio E, De La Orden V, Mediero-Valeros B, Navarro JL, et al. Oncological evaluation by positron-emission tomography, circulating tumor cells and alpha fetoprotein in patients with hepatocellular carcinoma on the waiting list for liver transplantation. Transpl Proc 2016;48(9):2962–2965.
11. Antonarakis ES, Lu C, Wang H, Luber B, Nakazawa M, Roeser JC, Chen Y, Mohammad TA, Chen Y, Fedor HL, et al. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. N Engl J Med 2014;371:1028–1038.
12. Campton DE, Ramirez AB, Nordberg JJ, Nordberg JJ, Drovetto N, Clein AC, Varshavskaya P, Friemel BH, Quarre S, Breman A, et al. High-recovery visual identification and single-cell retrieval of circulating tumor cells for genomic analysis using a dual-technology platform integrated with automated immunofluorescence staining. BMC Cancer 2015;15:360.
13. Dago AE, Stepansky A, Carlsson A, Luttgen M, Kendall J, Baslan T, Kolatkar A, Wigler M, Bethel K, Gross ME, et al. Rapid phenotypic and genomic change in response to therapeutic pressure in prostate cancer inferred by high content analysis of single circulating tumor cells. PLoS One 2014;9(8):e101777.
14. Frithiof H, Aaltonen K, Ryden L. A FISH-based method for assessment of *HER-2* amplification status in breast cancer circulating tumor cells following CellSearch isolation. Onco Targets Ther 2016;9:7095–7103.
15. Marchetti A, Del Grammastro M, Felicioni L, Malatesta S, Filice G, Centi I, De Pas T, Santoro A, Chella A, Brandes AA, et al. Assessment of EGFR mutations in circulating tumor cell preparations from NSCLC patients by next generation sequencing: Toward a real-time liquid biopsy for treatment. PLoS One 2014;9(8):e103883.
16. Ferreira MM, Ramani VC, Jeffrey SS. Circulating tumor cell technologies. Mol Oncol 2016;10(3):374–394.
17. Low WS, Wan Abas WA. Benchtop technologies for circulating tumor cells separation based on biophysical properties. Biomed Res Int 2015;2015:239362. https://doi.org/10.1155/2015/239362. Epub 2015 Apr 21.
18. Bhagat AA, Hou HW, Li LD, et al. Pinched flow coupled shear-modulated inertial microfluidics for high-throughput rare blood cell separation. Lab Chip 2011;11:1870–1878.
19. Desitter I, Guerrouahen BS, Benali-Furet N, Wechsler J, Jänne PA, Kuang Y, Yanagita M, Wang L, Berkowitz JA, Distel RJ, et al. A new device for rapid isolation by size and characterization of rare circulating tumor cells. Anticancer Res 2011;31(2):427–441.
20. Farace F, Massard C, Vimond N, Drusch F, Jacques N, Billiot F, Laplanche A, Chauchereau A, Lacroix L, Planchard D, et al. A direct comparison of CellSearch and ISET for circulating tumour-cell detection in patients with metastatic carcinomas. Br J Cancer 2011;105(6):847–853.
21. Adams DL, Zhu P, Makarova OV, Martin SS, Charpentier M, Chumsri S, Li S, Amstutz P, Tang CM. The systematic study of circulating tumor cell isolation using lithographic microfilters. RSC Adv 2014;9:4334–4342.
22. Xu L, Mao X, Imrali A, Syed F, Mutsvangwa K, Berney D, Cathcart P, Hines J, Shamash J, Lu YJ. Optimization and evaluation of a novel size based circulating tumor cell isolation system. PLoS One 2015;10(9):e0138032.
23. Che J, Yu V, Dhar M, Renier C, Matsumoto M, Heirich K, Garon EB, Goldman J, Rao J, Sledge GW, et al. Classification of large circulating tumor cells isolated with ultra-high throughput microfluidic Vortex technology. Oncotarget 2016;7:12748–12760.
24. Dhar M.; Pao E.; Renier C., Go D.E., Che J., Montoya R., Conrad R., Matsumoto M., Heirich K., Triboulet M., Rao J., Jeffrey S.S., Garon E.B., Goldman J., Rao N.P., Kulkarni R., Sollier-Christen E., di Carlo D. Label-free enumeration, collection and downstream cytological and cytogenetic analysis of circulating tumor cells. Sci Rep 2016;6:35474. https://doi.org/10.1038/srep35474.
25. Dhar M, Wong J, Che J, Matsumoto M, Grogan T, Elashoff D, Garon EB, Goldman JW, Christen ES, Di Carlo D, et al. Evaluation of PD-L1 expression on vortex-isolated circulating tumor cells in metastatic lung cancer. Sci Rep 2018;8:592.
26. Kidess-Sigal E, Liu HE, Triboulet MM, Triboulet MM, Che J, Ramani VC, Visser BC, Poultsides GA, Longacre TA, Marziali A, et al. Enumeration and targeted analysis of KRAS, BRAF and PIK3CA mutations in CTCs captured by a label-free platform: Comparison to ctDNA and tissue in metastatic colorectal cancer. Oncotarget 2016;7(51):85349–85364.
27. Liu HE, Triboulet M, Zia A, Vuppalapaty M, Kidess-Sigal E, Coller J, Natu VS, Shokoohi V, Che J, Renier C, et al. Workflow optimization of whole genome amplification and targeted panel sequencing for CTC mutation detection. Nat Genomic Med 2017;2:34.
28. Sinkala E, Sollier-Christen E, Renier C, Rosàs-Canyelles E, Che J, Heirich K, Duncombe TA, Vlassakis J, Yamauchi KA, Huang H, et al. Profiling protein expression in circulating tumour cells using microfluidic western blotting. Nat Commun 2017;8:14622.

29. Renier C, Pao E, Che J, Liu HE, Lemaire CA, Matsumoto M, Triboulet M, Srivinas S, Jeffrey SS, Rettig M, et al. Label-free isolation of prostate circulating tumor cells using Vortex microfluidic technology. Nat Precis Oncol 2017;1:15.

30. Lemaire C, Liu SZ, Wilkerson CL, Ramani VC, Barzanian NA, Huang KW, Che J, Chiu MW, Vuppalapaty M, Dimmick AM, et al. Fast and label-free isolation of circulating tumor cells from blood: From a research microfluidic platform to an automated fluidic instrument, VTX-1 Liquid Biopsy System. SLAS Technol 2018; 23(1):16–29. https://doi.org/10.1177/2472630317738698.

31. Sollier E, Go DE, Che J, Gossett DR, O'Byrne S, Weaver WM, Kummer N, Rettig M, Goldman J, Nickols N, et al. Size-selective collection of circulating tumor cells using Vortex technology. Lab Chip 2014;14:63–77.