Next-generation Liquid Biopsy Instruments: Challenges and Opportunities

Shu Zhu[1], Yaohui Fang[1], Kefan Guo[1], Zhonghua Ni[1], and Nan Xiang[1]

[1]Southeast University

June 2, 2022

Abstract

Conventional cancer diagnosis needs to excise diseased tissue from the patient's body for biopsy, causing severe injury to patients. Liquid biopsy with the superior advantage of minimal invasiveness has shown its ability to cancer diagnosis in real-time, and has been developing promising diagnostic instruments. However, until today, the developed instrument still cannot be an alternative to tissue biopsy in the majority of research and clinical settings. In this paper, we firstly summarize the challenges and limitations suffered by the existing liquid biopsy instrument. Then, the opportunities and future progression of the next-generation instrument are discussed in detail. In all, we hope that the future liquid biopsy instrument can be eventually integrated into the clinical workflow, and serve as a validated and reliable tool for cancer diagnosis.

Introduction

Cancer is a highly complex disease that is characterized by evolving pathophysiological processes [1]. Hence, treatment decisions are typically made according to the manifestation and progression of the primary tumor [2]. Currently, tissue biopsy technology, which excises or punctures diseased tissue from the patient for pathological examination, is considered the "gold standard" for cancer diagnosis and treatment [3]. Nevertheless, as various tumor entities originate from internal organs, acquiring diseased tissue can be highly risky and challenging. Furthermore, clones differentiate into various subpopulations during the biological process of tumorigenesis, resulting in tumor heterogeneity [4,5]. This diversity and plasticity cannot be detected in single-tissue biopsy samples owing to their spatial and temporal heterogeneity, making it difficult to acquire comprehensive information on the genetic and epigenetic variability of cancer [6].

Compared to tissue biopsy, liquid biopsy (LB) is a minimally invasive diagnostic tool that is less wound-inducing and can be conducted frequently, allowing longitudinal monitoring of the dynamic changes in tumors and thereby its malignancy [7]. Thus, a straightforward assessment of tumor metastases and the biochemical changes during blood-borne cancer dissemination becomes possible by cell or molecular monitoring. In contrast to tissue biopsy, LB mainly involves analyzing circulating tumor cells (CTCs), circulating cell-free nucleic acids, and exosomes from the peripheral blood of patients with cancer (Fig. 1) [8,9]. CTCs are cancer cells that actively or passively escape from the primary tumor site to the bloodstream and are regarded as the culprits of cancer metastasis. CTC analysis has the potential to provide disease-related information on tumor composition, invasiveness, drug susceptibility, and treatment resistance [10,11]. Circulating cell-free nucleic acids, which mainly comprise circulating cell-free DNA (cfDNA), are typically discharged from apoptotic and necrotic tumor cells into the bloodstream [12]. Most cfDNA originates from non-malignant cells, whereas circulating tumor DNA (ctDNA) derived from tumor cells typically accounts for 0.1–10% of the total cfDNA, depending on the tumor burden, inflammatory status, cellular turnover, and accessibility of cancer cells to the blood vessels [13]. Therefore, cell-free nucleic acid analysis can provide detailed information relevant to cancer diagnosis and therapy [14]. Exosomes are extracellular vesicles secreted by healthy and tumor cells, containing proteins, RNA, and DNA from patients' tumors [15]. Thus, tumor-derived exosomes can indicate

the nature and status of tumor entities, as they contain all cellular contents originating from their tumor cells.

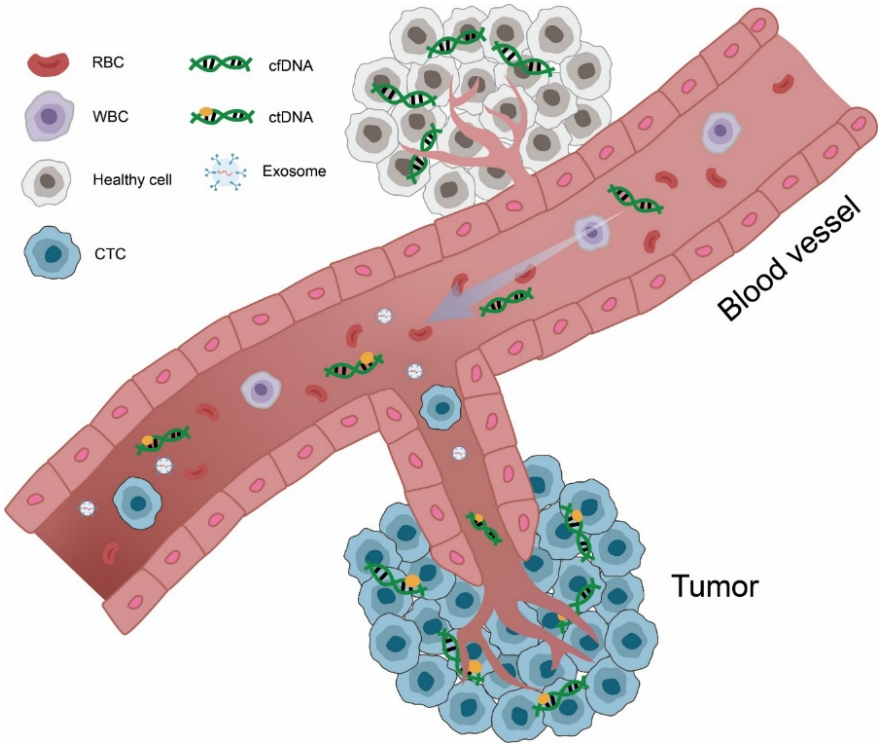

Fig. 1. Source of liquid biopsy tumor material. Solid tumor masses may shed circulating tumor cell (CTC), circulating cell-free nucleic acids (ctDNA), and exosome into the bloodstream.

Several instruments have been developed to achieve optimal LB assessment (Fig. 2**,** Table 1). CellSearch (Veridex, USA), the first instrument approved by the United States Food and Drug Administration as an instrument for separating and detecting CTCs in LB, has been widely used for breast, colorectal, and prostate cancer diagnosis. Using this instrument, CTCs are separated from blood cells by magnetic nanoparticles coated with anti-epithelial cellular adhesion molecule (EpCAM) antibodies [16]. Immunofluorescence analysis is then performed on the separated cells for CTC characterization. This approach provides a CTC detection specificity of $> 99\%$ and sensitivity of $> 97\%$. As the detection of circulating cell-free nucleic acids depends on molecular biology techniques that are complex to operate and require additional analytical platforms (such as quantitative polymerase chain reaction [qPCR], BEAMing, Safe-SeqS, and CAPP-Seq) [9,17], LB-based instruments are commonly used to extract cell-free nucleic acids. For example, the KingFisher Flex instrument has been applied for cfDNA extraction from blood samples based on the magnetic enrichment technique [18], wherein cfDNA becomes positively charged by magnetic beads that bind to the negatively charged phosphate DNA backbone. For exosome analysis, serial centrifugation or ultracentrifugation steps are performed to eliminate nanoscale contaminants [19]. However, these methods require time-consuming sample preparation protocols and cannot achieve high purity or high-throughput analysis. To address these limitations, an instrument named EXODUS was developed based on a novel ultrafiltration strategy [20]. In this instrument, exosomes are filtered through nanoporous membranes, and double-coupled harmonic oscillations are introduced to these membranes to generate transverse waves to inhibit fouling effects, resulting in enhanced processing speed, yield, and purity.

Nowadays, although many LB instruments have been proposed for cancer diagnosis, most of them still exist a wide gap between the laboratory devices and the commercialization of instrument. According to

the differences of working mechanism and application object, the developed instruments also face various limitations. In this article, the challenges of the existed LB instrument, as well as the opportunities for next-generation LB instruments are discussed, aiming at improving the sensitivity, efficiency, and accuracy of cancer diagnosis.

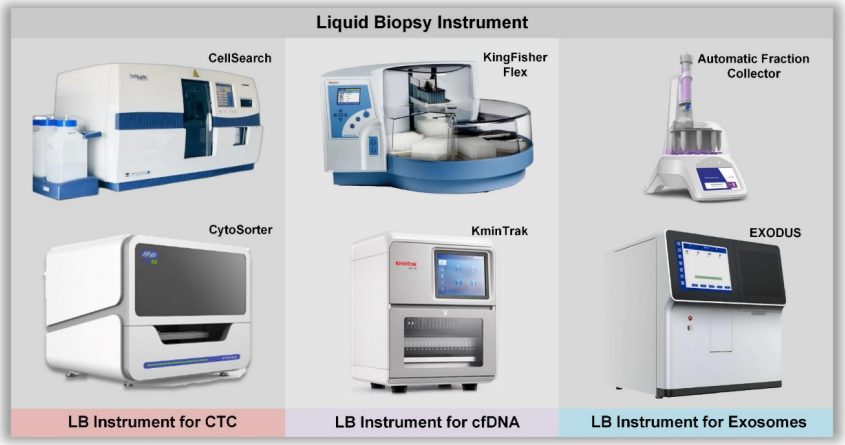

Fig. 2. Representative liquid biopsy (LB) instruments for isolating or analyzing circulating tumor cells (CTCs), circulating cell-free nucleic acids, or exosomes.

Table 1. Summary of the representative liquid biopsy instruments

Instrument	Object	Isolation method	Detection method	Application	Reference
CellSearch	CTC	Immunomagnetic separation	Fluorescence	Cancers such as breast, colorectal, and prostate	[16]
Parsortix	CTC	Cross-flow filtration	N/A	Castration resistant prostate cancers	[25]
ClearCell FX	CTC	Inertial microfluidics	N/A	Lung, head and neck, and non-small-cell lung cancers	[26]
KingFisher Flex	cfDNA	Immunomagnetic separation	N/A	Cancers such as pancreatic, colorectal, and non-small-cell lung cancers	[18]
KminTrak	cfDNA	Immunomagnetic separation	N/A	Gastric cancer	[27]
Automatic Fraction Collector	Exosomes	Centrifugation and filtration	N/A	Colorectal, head and neck, and ovarian cancers	[28]

Instrument	Object	Isolation method	Detection method	Application	Reference
EXODUS	Exosomes	Filtration	N/A	Kidney and bladder cancers	[20]

Can existing liquid biopsy instrument replace tissue biopsy?

Conventional tissue biopsy, as the key of the diagnostic pathology, can make a definite histopathological diagnosis over the vast majority of the cancer cases [21]. However, tissue biopsy not only needs complex, time-consuming and expensive operation processes, but also is difficult to obtain pathological tissue from body organ. In addition, the conventional tissue biopsy cannot be used to monitor the treatment response. In contrast, the liquid biopsy instrument can be easily applied to interrogate cancer-related biomarkers sustainably with minimal invasive, and then achieve real-time monitor for cancers.

The major challenge of existing LB instruments is their insufficient accuracy and sensitivity caused by the heterogeneity and rarity of cancer biomarkers [22]. To address these issues, LB instruments used for CTC analysis typically contain white blood cell (WBC) depletion modules, which employ immune- or physical isolation methods depending on the morphological, biological, or physical properties of the CTC and WBCs. The immune-isolation method mainly allows the positive selection of CTCs through antigens expressed in epithelial cells, such as EpCAM. However, subpopulations of metastatic tumor cells with low EpCAM expression or undergoing epithelial-to-mesenchymal transition (EMT) cannot be captured or identified using this method [23]. Physical isolation methods typically separate CTCs based on the differences in size, shape, or deformability between CTCs and blood cells. However, CTC subpopulations with physical features similar to those of WBCs cannot be differentiated or sorted during the separation process [24]. Furthermore, the LB instrument used for cfDNA also faces similar limitations. The ctDNA originating from CTCs can be contaminated by those derived from normal blood cells of the patient and might only account for ˜0.01% of the total cfDNA. Various patient-related factors, including pregnancy, smoking, exercise, and various non-malignant conditions, also affect the cfDNA levels in blood [13]. Therefore, the association between patient-related factors and the performance of specific cfDNA assays may not be entirely accurate.

The second challenge of the existing LB instruments is their inadequate automation and integration. To achieve rapid and real-time cancer diagnosis, LB instruments have been developed to reduce biochemical operations, lower the economic burden for training hospital personnel, including pathologists, nurses, and physicians, and simplify the interpretation of the test results. However, most LB instruments can only isolate biomarkers and are unequipped to conduct necessary subsequent analyses. For example, the Parsortix and ClearCell FX platforms are designed for CTC isolation [25,26], whereas the KminTrak instrument is used for cfDNA extraction [27]. The Automatic Fraction Collector can only serve as a pretreatment unit for exosome isolation [28]. Furthermore, most LB instruments require complex biochemical operations, even during the biomarker isolation process, which further impairs their automation levels. For example, CytoSorter requires labor-intensive immunoaffinity operations to separate CTCs from blood cells [29]. For LB instruments without an analytical module, the analysis of biomarkers commonly relies on complex biochemical operations, such as fluorescent in situ hybridization (FISH), next-generation sequencing (NGS), and digital PCR (dPCR) [30]. For instance, for cfDNA detection, PCR technologies are typically used to amplify the target DNA fragment and improve analytical sensitivity. These assays are usually difficult to integrate into the corresponding LB instrument [31].

The third challenge is the validity of the detection results. CellCollector and KminTrak [32,33] as new-developed LB instruments can isolate CTCs and cfDNA, separately. Although the number of CTCs in the blood and the ctDNA content in plasma have been used as a reference for early treatment response of cancers, they do not inform patients about the cancer progression stage, as no additional clinical evidence demonstrating their diagnostic efficacy is available compared to standard imaging-based tools [34]. Thus,

CTC- and ctDNA-based LB technologies are not sophisticated enough to monitor treatment response in the clinical setting [35]. In addition, early-stage cancer detected via LB instrument may be indolent or will never develop into life-threatening cancers, such as in the case of prostate and breast cancers. This may result in the overdiagnosis of incidental cancers that are unlikely to affect the overall health or lifespan of the patients [36]. Notably, the overtreatment guided by an overdiagnosis can have considerable consequences [4]. For example, complications that include incontinence or erectile dysfunction can be aroused due to overtreatment in prostate cancers.

To better understand the evolutionary changes in the genetic and epigenetic landscape of tumors, multigene assays of biomarkers obtained from LB instruments are often performed. However, these additional testing require a considerable investment of time, knowledge, and resources, such as state-of-the-art NGS machines, adequate storage space for multigene sequencing data, and a wet laboratory [37]. National and international central laboratories are better equipped to perform these assays, wherein the large-scale sample processing would be more economical. Furthermore, conducting multigene assays rather than single assays may not be worthwhile for local pathology laboratories. Therefore, LB instruments combined with multigene assays for cancer detection are difficult to apply in point-of-care (POC) settings.

Although LB instruments can significantly reduce the investment in manual operation and repeated minimally invasive detection of cancers, they have been commercially developed to a much lesser extent than conventional tissue biopsy approaches. In addition, the rapid development of tissue biopsy- and imaging-based diagnosis methods make them more accessible to researchers and clinicians than LB instruments. Considering the limitations of the existing LB instruments, an obvious challenge for broad clinical applications should be addressed. Therefore, the current LB instrument is not sophisticated enough to replace tissue biopsy.

What's the opportunity of next-generation liquid biopsy instrument?

Until today, the accuracy of liquid biopsy is not comparable with that of tissue biopsy in cancer confirmation, as well as their clinical utility is not well demonstrated, thus current liquid biopsy only serves as a complementary method to tissue biopsy [38]. To fully benefit from the many advantages of LB, next-generation LB instruments must become a priority.

First, enhanced detection accuracy and sensitivity are warranted; most existing LB instruments for cancer diagnosis are only based on a single biomarker, thereby significantly limiting their output. Comprehensive detection using multiple biomarkers might address this issue and is crucial for the advancement of next-generation LB instruments [39]. For example, CTC and ctDNA detection are believed to be complementary, with ctDNA being more suitable for detecting mutations and CTCs for studying drug sensitivity and tumor heterogeneity [40]. Notably, achieving multiple biomarker-based detections should first address key issues, such as integrating different biomarker isolation components in a single instrument.

Second, most LB instruments still require several manual operation steps, such as cell incubation and immunofluorescence labeling, resulting in multiple variables that can cause substantial errors in their clinical utility. Additionally, regarding some comprehensive sample-to-result workflows, the isolation and detection of biomarkers must be performed across platforms. For example, in the LiquidBiopsy platform workflow [31,41], cfDNA is purified and analyzed on the LiquidBiopsy, and Ion Chef and Ion S5 XL platforms, respectively. Integrating the preparation, isolation, detection, and other components into a single LB instrument can improve its automation level and simplify the operation steps, facilitating the standardization of crucial procedures, thereby improving detection reliability. Therefore, ideal next-generation instruments should meet the requirement that even non-professionals can readily obtain reliable results using highly automated LB instruments.

Multiple analytical components are hard to integrate with other LB instruments, especially multigene assays that require considerable sample processing time, as well as expensive and bulky equipment [42]. To reduce the complexity of LB instruments, complex biochemical assays can be performed in a superior laboratory. Therefore, the next-generation LB instrument may be designed to isolate and then primarily detect

biomarkers, and a sophisticated follow-up analysis may be conducted through off-site centralized testing. Furthermore, a database should be established to share extensive analytical data in real-time, which may accelerate the investigation of cancers. In the future, integrating label-free based cell separation and detection components in the LB instrument would make it possible to achieve this goal. With such LB instruments, CTCs may be isolated based on their physical properties using inertial microfluidic, acoustofluidics, or other technologies [43,44]. Then, isolated CTCs may be detected by label-free interrogation technologies, including impedance cytometry and image flow cytometry [45,46]. The obtained CTCs with high activeness will be suitable for further investigation of tumor composition, invasiveness, drug susceptibility, and resistance to therapy in a central laboratory [47]. Moreover, a label-based biomarker separation method that obtains active target biomarkers by labeling non-target biomarkers, as well as impedance cytometry for detecting submicron bioparticles (such as exosomes), also holds promise for rapid and primarily detection of biomarkers [48,49].

Assessment of multiple biomarkers and parameters for the comprehensive detection of cancer, which will generate a large amount of analytical data, may be the future of LB instruments. Hospital personnel who obtain these data should receive adequate training to interpret such results and act accordingly. However, such training typically requires considerable time and effort. Moreover, with the increase in detection parameters, the corresponding analytical data also become complex, causing obstacles for physicians to make accurate diagnoses and treatment decisions. In next-generation LB instruments, artificial intelligence may help comprehensively analyze the clinicogenomic, metabolomic, immunomic, microbiomic, and homeostatic data obtained from LB instruments to assist diagnosis and treatment decisions [50].

Apart from making next-generation LB instruments more multifunctional, downscaling the size of instruments may also be a significant future development [51,52]. In general, patients experience a recovery period of months to years after cancer surgery, and daily monitoring is essential for preventing cancer recurrence. However, LB-based total-analysis instruments may not be easily accessible to patients in health resource-limited locations. Therefore, the development of portable or wearable LB instruments for real-time monitoring of cancer is of great significance, as critical detection data can be obtained and shared with physicians in real-time to evaluate the recovery of patients with cancer. Label-free cfDNA and CTC detection methods or membrane filtration methods, such as electrochemical sensing technology for label-free cfDNA detection and thin membrane with special micro-pores or structures for tumor cell line capture [53,54], are promising approaches to achieve this requirement, as no expensive reagents or bulky external platforms are required. Portable or wearable LB instruments are expected to be developed to isolate and detect tumor biomarkers from patients.

Several points need to be considered to make next-generation LB instruments commercially available. First, the basic requirement of the LB instrument is to ensure analytical and clinical validity, and achieve clinical utility. Additionally, cost-effectiveness analyses are essential, as an LB instrument can be highly expensive and inaccessible to certain clinical settings. Lastly, the analytical results must be incorporated into the clinical workflow to guide cancer diagnosis and treatment. Before being widely adopted in clinical applications, a large number of on-site validations should be performed in clinical trials by comparing and evaluating the results of the newly developed LB instruments and routine assays [55].

In all, with the improvement of highly sensitive techniques for accuracy disease detection, the next-generation liquid biopsy instruments are a promising tool for the early diagnosis, real-time monitor, prognosis, and prediction of the response to treatment in various types of cancers.

Conclusion

Comparing to conventional tissue biopsy that excises diseased tissue from the patient's body, liquid biopsy with the advantages of minimal invasiveness can be used to rapid and real-time diagnosis of cancers, as well as the longitudinal monitoring along tumor dynamic changes, and thus many liquid biopsy-based instruments emerged. However, existing liquid biopsy instruments suffer many limitations, such as insufficient accuracy and sensitivity, inadequate automation, poor commercialization, etc., thus existing instrument still cannot

be an alternative to tissue biopsy. The ideal LB instrument either allows precise, reliable, rapid, and comprehensive cancer detection in the laboratory or can be used as a portable real-time monitoring tool in a health resource-limited place. With the improvement of highly sensitive techniques for precision medicine, next-generation LB instruments are promising tools for early diagnosis, real-time monitoring, prognosis, and prediction of response to treatment for various cancer types. We hope that the future LB instrument will become an optimal and reliable technology upon which we can all rely.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This research work is supported by the National Natural Science Foundation of China (81727801 and 51875103), the Natural Science Foundation of Jiangsu Province (BK20190064), and the Scientific Research Foundation of Graduate School of Southeast University (YBPY2104).

Data availability: The data that supports the findings of this study are available from the corresponding author upon reasonable request.

Reference

[1] Corcoran, R. B., *Nature Medicine* 2020, *26* , 1815-1816.

[2] Battat, S., Weitz, D. A., Whitesides, G. M., *Lab on a Chip* 2022, *22* , 530-536.

[3] Gronholm, M., Feodoroff, M., Antignani, G., Martins, B., Hamdan, F., Cerullo, V., *Cancer Research* 2021, *81* , 3149-3155.

[4] Ignatiadis, M., Sledge, G. W., Jeffrey, S. S., *Nature Reviews Clinical Oncology* 2021, *18* , 297-312.

[5] Taftaf, R., Liu, X., Singh, S., Jia, Y. Z., Dashzeveg, N. K., Hoffmann, A. D.*, et al.* , *Nature Communications* 2021,*12* , 4867.

[6] Parikh, A. R., Leshchiner, I., Elagina, L., Goyal, L., Levovitz, C., Siravegna, G.*, et al.* , *Nature Medicine* 2019,*25* , 1415–1421.

[7] Jeffrey, S. S., Toner, M., *Lab on a Chip* 2019, *19* , 548-549.

[8] Laraib, U., Sargazi, S., Rahdar, A., Khatami, M., Pandey, S.,*International Journal of Biological Macromolecules* 2022,*195* , 356-383.

[9] Pinzani, P., D'Argenio, V., Del Re, M., Pellegrini, C., Cucchiara, F., Salvianti, F.*, et al.* , *CLINICAL CHEMISTRY AND LABORATORY MEDICINE* 2021, *59* , 1181-1200.

[10] Wong, B. S., Shah, S. R., Yankaskas, C. L., Bajpai, V. K., Wu, P. H., Chin, D.*, et al.* , *Nature Biomedical Engineering*2021, *5* , 26-40.

[11] Neumann, M. H. D., Bender, S., Krahn, T., Schlange, T.,*Computational and Structural Biotechnology Journal* 2018,*16* , 190-195.

[12] Petranovic, M., Raoof, S., Digumarthy, S. R., Sharma, A., Shepard, J. A. O., Gainor, J. F.*, et al.* , *Journal of the American College of Radiology* 2022, *19* , 336-343.

[13] Fernandez-Lazaro, D., Hernandez, J. L. G., Garcia, A. C., Martinez, A. C., Mielgo-Ayuso, J., Cruz-Hernandez, J. J.,*Diagnostics* 2020, *10* , 215.

[14] Gorgannezhad, L., Umer, M., Islam, M. N., Nguyen, N. T., Shiddiky, M. J. A., *Lab on a Chip* 2018, *18* , 1174-1196.

[15] Kosaka, N., Kogure, A., Yamamoto, T., Urabe, F., Usuba, W., Prieto-Vila, M., *et al.* , *Experimental and Molecular Medicine* 2019, *51* , 1-9.

[16] Schuster, E., Taftaf, R., Reduzzi, C., Albert, M. K., Romero-Calvo, I., Liu, H. P., *Trends in Cancer* 2021, *7* , 1020-1032.

[17] Michela, B., *Diagnostics* 2021, *11* , 1391.

[18] Yin, L. D., Pu, N., Thompson, E., Miao, Y., Wolfgang, C., Yu, J., *Clinical Cancer Research* 2021, *27* , 740-748.

[19] Li, G. Y., Tang, W. W., Yang, F., *Biotechnology Journal*2020, *15* , 1900225.

[20] Chen, Y. C., Zhu, Q. F., Cheng, L. M., Wang, Y., Li, M., Yang, Q. S., *et al.* , *Nature Methods* 2021, *18* , 212-218.

[21] Wan, J. C. M., Massie, C., Garcia-Corbacho, J., Mouliere, F., Brenton, J. D., Caldas, C., *et al.* , *Nature Reviews Cancer*2017, *17* , 223-238.

[22] Miccio, L., Cimmino, F., Kurelac, I., Villone, M. M., Bianco, V., Memmolo, P., *et al.* , *View* 2020, *1* , 20200034.

[23] Pantel, K., Alix-Panabieres, C., *Cancer Research* 2013,*73* , 6384-6388.

[24] Tang, W. L., Zhu, S., Jiang, D., Zhu, L. Y., Yang, J. Q., Xiang, N., *Lab on a Chip* 2020, *20* , 3485-3502.

[25] Miller, M. C., Robinson, P. S., Wagner, C., O'Shannessy, D. J.,*Cytometry Part A* 2018, *93a* , 1234-1239.

[26] Zhang, X. J., Zhu, Z. X., Xiang, N., Long, F. F., Ni, Z. H.,*Analytical Chemistry* 2018, *90* , 4212-4220.

[27] Yu, P., Zhu, S., Luo, Y., Li, G., Pu, Y., Cai, B., *et al.* , *Journal of Oncology* 2022, *2022* , 9635218.

[28] Freitas, D., Balmana, M., Pocas, J., Campos, D., Osorio, H., Konstantinidi, A., *et al.* , *Journal of Extracellular Vesicles* 2019, *8* , 1621131.

[29] Jin, L. D., Zhao, W. H., Zhang, J., Chen, W. J., Xie, T., Wang, L. B., *et al.* , *Cancer Medicine* 2020, *9* , 1638-1647.

[30] Castro-Giner, F., Gkountela, S., Donato, C., Alborelli, I., Quagliata, L., Ng, C. K. Y., *et al.* , *Diagnostics* 2018,*8* , 31.

[31] Takeda, K., Yamada, T., Takahashi, G., Iwai, T., Ueda, K., Kuriyama, S., *et al.* , *Cancer Science* 2019, *110* , 3497-3509.

[32] Dizdar, L., Fluegen, G., Dalum, G., Honisch, E., Neves, R. P., Niederacher, D., *et al.* , *Molecular Oncology* 2019,*13* , 1548-1558.

[33] Chen, Y., Zhou, Z., Zhu, S., Ni, Z., Xiang, N.,*Microchem. J.* 2022, *177* , 107284.

[34] Pashayan, N., Pharoah, P. D. P., *Science* 2020,*368* , 589-590.

[35] Dawson, S. J., Tsui, D. W. Y., Murtaza, M., Biggs, H., Rueda, O. M., Chin, S. F., *et al.* , *New England Journal of Medicine* 2013, *368* , 1199-1209.

[36] Lennon, A. M., Buchanan, A. H., Kinde, I., Warren, A., Honushefsky, A., Cohain, A. T., *et al.* , *Science* 2020,*369* , eabb9601.

[37] van de Haar, J., Hoes, L., Voest, E., *Esmo Open* 2019,*4* , e000516.

[38] Scheerens, H., Malong, A., Bassett, K., Boyd, Z., Gupta, V., Harris, J., *et al.* , *Cts-Clinical and Translational Science*2017, *10* , 84-92.

[39] Kaiser, J., *Science* 2018, *359* , 259.

[40] Schweizer, M. T., Antonarakis, E. S., *Science Translational Medicine* 2015, *7* , 312fs345.

[41] Chen, W. R., Zhang, J. C., Huang, L. J., Chen, L., Zhou, Y. L., Tang, D. J.*, et al.* , *Clinical Breast Cancer* 2019,*19* , E239-E246.

[42] Li, B. T., Janku, F., Jung, B., Hou, C., Madwani, K., Alden, R.*, et al.* , *Annals of Oncology* 2019, *30* , 597-603.

[43] Li, J. F., Shen, C., Huang, T. J., Cummer, S. A., *Science Advances* 2021, *7* , eabi5502.

[44] Xiang, N., Ni, Z. H., *Lab on a Chip* 2022, *22* , 757-767.

[45] Blasi, T., Hennig, H., Summers, H. D., Theis, F. J., Cerveira, J., Patterson, J. O.*, et al.* , *Nature Communications* 2016,*7* , 10256.

[46] Zhu, S., Zhang, X. Z., Chen, M., Tang, D. Z., Han, Y., Xiang, N.*, et al.* , *Analytica Chimica Acta* 2021, *1175* , 338759.

[47] Gwak, H., Kim, J., Cha, S., Cheon, Y. P., Kim, S. I., Kwak, B.*, et al.* , *Biomicrofluidics* 2019, *13* , 024113.

[48] Han, Z. W., Wan, F. N., Deng, J. Q., Zhao, J. X., Li, Y. K., Yang, Y. J.*, et al.* , *Nano Today* 2021, *38* , 101203.

[49] Xia, H. M., Wu, J. W., Zheng, J. J., Zhang, J., Wang, Z. P.,*Lab on a Chip* 2021, *21* , 1241-1268.

[50] Liu, C., Sun, J. S., *Annual Review of Analytical Chemistry* 2021, *14* , 1-19.

[51] Jiang, F., Xiang, N., *Anal. Chem.* 2022, *94* , 1859-1866.

[52] Kim, T. H., Wang, Y., Oliver, C. R., Thamm, D. H., Cooling, L., Paoletti, C.*, et al.* , *Nature Communications* 2019,*10* , 1478.

[53] Furniturewalla, A., Chan, M., Sui, J., Ahuja, K., Javanmard, M., *Microsystems & Nanoengineering* 2018, *4* , 20.

[54] Li, R. Y., Liu, L., Bei, H. X., Li, Z. J., *Biosensors & Bioelectronics* 2016, *79* , 457-466.

[55] Xu, H. Y., Baidoo, A. A. H., Su, S. S., Ye, J. R., Chen, C. S., Xie, Y. P.*, et al.* , *Translational Lung Cancer Research*2019, *8* , 135-143.