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Liquid biopsy of circulating tumor cells: From isolation, enrichment, and genome sequencing to clinical applications

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Summary. Circulating tumor cells (CTCs), shed from primary tumors into the bloodstream, play a crucial role in metastasis and hold great potential in cancer diagnosis, prognosis, and treatment monitoring. Conventional CTC detection using epithelial biomarkers like epithelial cell adhesion molecule (EpCAM) for immunocapture overlooks mesenchymal-like CTCs with high metastatic potential, spurring the development of non-immunocapture technologies that use biophysical traits for enrichment. Innovations in microfluidic platforms and multi-parametric sorting improve isolation efficiency and address related challenges. Breakthroughs in single-cell genomic and transcriptomic sequencing enable in-depth molecular characterization of CTCs. Clinically, CTC enumeration and molecular profiling are emerging as real-time tools for assessing therapeutic response and predicting outcomes, especially in metastatic breast, prostate, and colorectal cancers. This review focuses on CTC isolation, enrichment techniques, their applications in different tumors, downstream analysis progress, and potential in precision medicine.

Key words: Circulating tumor cells, Isolation, Enrichment, Sequencing, Characterization, Clinical applications

Introduction

Cancer metastasis is one of the major causes of death among cancer patients, and circulating tumor cells (CTCs) play a crucial role in this process. CTCs detach from primary tumors or metastases and enter the bloodstream (Auwal et al., 2024). They not only cause distant metastases but also show great potential as biomarkers in cancer diagnosis, prognosis assessment, and treatment monitoring (Hou et al., 2019).

In recent years, significant progress has been made in CTC research. Although the concept was proposed over a century ago, in-depth research has only become possible recently with the help of advanced technologies. Early CTC research mainly focused on the detection and quantification of CTCs in the blood. However, the scope of research has now expanded to include enrichment, genomic analysis, and clinical application exploration. The traditional CellSearch system uses epithelial cell adhesion molecule (EpCAM) for immunocapture to predict the prognosis of metastatic cancer. However, this method cannot detect mesenchymal-like CTCs with high metastatic potential (Franken et al., 2023). This limitation has spurred the development of non-immunocapture technologies, such as microfluidic-and inertial-based separation techniques. These technologies can capture a wider range of CTCs by utilizing their biophysical properties. In addition, the application of microfluidic platforms, nanotechnology, and multi-parameter sorting strategies has effectively improved the isolation efficiency and purity of CTCs. Analyzing the genomes and transcriptomes of isolated CTCs, especially through single-cell sequencing, can reveal information such as gene mutations, copy number variations, and gene expression profiles, which is of great significance for a deeper understanding of tumor metastasis mechanisms and drug resistance (Yang et al., 2021).

In clinical applications, CTCs have potential value in cancer management. In the early-diagnosis stage, new technologies based on digital PCR and others are emerging, improving the detection sensitivity of CTCs. During the treatment process, changes in the number and characteristics of CTCs can reflect the treatment effect. A high CTC count often indicates a poor prognosis for

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patients, especially in metastatic cancers (Geng et al., 2022). However, CTC research still faces many challenges. Nowadays, there is a lack of standardization in related techniques, making it challenging to unify and contrast the detection outcomes of different laboratories. The biological mechanisms of CTCs have not been fully elucidated, limiting in-depth understanding of their functions. Moreover, translating research findings into clinical practice is fraught with difficulties and requires overcoming many obstacles.

This review aimed to conduct a comprehensive summary of the research regarding CTCs. It encompasses CTC isolation and enrichment techniques, advancements in downstream analysis, as well as their potential significance in precision medicine. Through in-depth exploration of these aspects, it offers a reference for the further research and clinical applications of CTCs and contributes to the development of the cancer diagnosis and treatment domain.

Characteristics of CTCs

CTCs exhibit distinctive characteristics. Morphologically, CTCs are larger than normal cells, with large and irregular nuclei and a high nucleus-to-cytoplasm ratio, indicating enhanced metabolic and proliferative potential (Auwal et al., 2024). They also have poor deformability, which is related to changes in the cytoskeleton (Takagi et al., 2020). Numerically, although the detection rate of CTCs in metastatic cancer patients is higher than that in early-stage cancer, the content in blood remains extremely low (typically only a few to several dozen cells per milliliter), such as the positive threshold of ≥ 5/7.5 mL for advanced breast cancer (Yang et al., 2021). CTCs overlap with white blood cells in size and other biophysical properties, making it difficult to isolate CTCs from the blood (Lin et al., 2021).

CTCs also exhibit a high degree of heterogeneity. They can present epithelial, mesenchymal, or mixed phenotypes (Loeian et al., 2019). Most CTCs derived from solid cancers retain the expression of EpCAM on the plasma membrane, but many CTCs lack EpCAM, such as those from non-epithelial cancers (melanoma and sarcoma) (Lu et al., 2019), CTCs that have undergone epithelial-mesenchymal transition (EMT), or "stealth" CTCs coated by platelets. At the molecular level, CTCs vary in gene mutations, copy number variations, and gene expression, resulting in different responses to targeted therapies (Rossi et al., 2022).

CTC isolation and enrichment

The isolation and enrichment of CTCs represent essential initial stages in harnessing their potential as biomarkers for cancer diagnosis, prognosis evaluation, and treatment surveillance. Considering the exceedingly low concentration of CTCs in the bloodstream and the multitude of challenges presented by their resemblance to other blood cells, a variety of techniques have been devised for their isolation and enrichment. These methods can be broadly categorized into immunocapture-based and non-immunocapture-based methods. Each method has its own advantages and disadvantages (Table 1) and relies on the physical and biological properties of CTCs, including size, density, cell charge, and the expression of cell markers, among others (Fig. 1).

Non-immunocapture methods

Size and deformability-based isolation and enrichment

Technologies for the separation and enrichment of CTCs based on size mainly include microfiltration and microfluidic device technologies. Microfiltration technology takes advantage of the fact that CTCs are larger than most normal blood cells. A blood sample containing CTCs is passed through a microfiltration membrane with a specific pore size, trapping the CTCs while allowing small blood cells to pass through. Take the ScreenCell system, for example, which is founded on the microfiltration principle. By capitalizing on the characteristic that CTCs are larger than most normal blood cells, it uses a microfiltration membrane with a definite pore size to carry out the separation of CTCs (Lopresti et al., 2022).

Microfluidic devices, on the other hand, are based on the characteristic that CTC deformability is usually lower than that of normal blood cells. A blood sample is allowed to flow through a microchannel with narrow passages. Blood cells with high deformability can pass through, while CTCs with poor deformability are trapped or guided for separation. For example, the Parsortix system is based on a microfluidic chip (Obermayr et al., 2023). With its special microchannels and obstacles, it captures CTCs according to the size and deformability of cells, and can also stain and analyze the captured cells. However, the deformability of CTCs is affected by cell status and tumor heterogeneity, resulting in the ineffective separation of some CTCs.

Density-based isolation and enrichment

Density-based enrichment methods mainly rely on the density differences between CTCs and other cells in the blood for separation. The commonly used density-based separation technique is density gradient centrifugation. In this method, a medium with a gradient density, such as Ficoll-Hypaque (Bartkowiak et al., 2025), needs to be prepared first. The collected blood sample is carefully layered on top of the density gradient medium and then centrifuged. Under the action of centrifugal force, cells of different densities will move to their corresponding positions in the gradient medium according to their densities. Red blood cells, due to their high density, will settle at the bottom of the centrifuge tube; white blood cells, with a slightly lower density,

Table 1. Methods for the isolation and enrichment of CTCs, corresponding examples, and their advantages and disadvantages.

Subcategory		Examples	Advantages	Disadvantages
Non-immunocapture methods	Size and deformability-based isolation and enrichment	ISET, ScreenCell, Circulogix FaCTChecker, ANGLE Parsortix, Vortex Biosciences VTX-1, ClearCell, GEDI chip, GEM chip, CTC-iChip, FMSA, MCA, CTC-FIND, FAST, DLD, CellSieve, iDEP	No reliance on surface markers, capable of detecting CTC clusters, with relatively low cost, preserving cell viability (Rzhevskiy et al., 2025).	Low purity, unstable detection efficiency (Rzhevskiy et al., 2025), difficulty in standardization (Lin et al., 2021).
	Density-based isolation and enrichment	Ficoll-Paque, OncoQuick, RosetteSep, RareCyte (AccuCyte-CyteFinder)	Simple operation, low cost, ability to handle large samples (Lin et al., 2021), preservation of cell viability and compatibility, compatibility with other technologies (Yang et al., 2021).	Low purity and unstable efficiency, large differences in recovery rates (Saini et al., 2025), omission of small CTCs and specific sub-populations (Hendricks et al., 2020).
	Charge/electrical field force-based isolation and enrichment	ApoCell ApoStream, DEPArray, iDEP, FACS	Label-free or selective labeling (Lin et al., 2021), maintenance of cell viability and plasticity, High sensitivity and specificity (Yang et al., 2021), adaptation to heterogeneous CTCs.	Dependence on equipment and high cost, low throughput and time-consuming, loss of small-sized CTCs, necessity of pre-enrichment, potential technical limitations (Auwal et al., 2024).
	Photoacoustic effect-based isolation and enrichment	Cytophone	Ultra-high sensitivity, non-invasive detection, label-free operation, real-time dynamic monitoring, detection of multiple types of signals, high specificity (Galanzha et al., 2019).	Limited scope of application, technical dependence, limitation of vascular depth (Galanzha et al., 2019).
	Microfluidic-based isolation and enrichment	ClearCell, CTC-Chip, IsoFlux System, HB-chip, GEM chip, GO chip, BioFluidica Modular Sinusoidal Microsystem, NanoVelcro, MagRC, CTC-iChip, GEDI chip, OncoCEE, LiquidBiopsy, Ephesia, BioFluidica/Modular Sinusoidal Microsystem, MagRC, rare-cell scWB (Milo), DEPArray, FMSA, online integrated inertial-magnetophoresis microfluidic chip-ICP-MS system, GEM chip, Microfluidic Western blot, ClearCell, ApoCell ApoStream, ANGLE Parsortix, TetherChip, DLD, iDEP, FAST	High sensitivity and recovery rate, compatibility with downstream analysis (Descamps et al., 2022), label-free separation (Loeian et al., 2019), high throughput and rapid processing, low contamination and miniaturization.	High cost and instrument complexity, inadequate handling of CTC heterogeneity.
Immunocapture methods	Immunocapture positive isolation and enrichment	Cellsearch, Nanovelcro, GILUPI CellCollector, MagWIRE, IVFC, AdnaTest, Target Selector, MagSweeper, DEPArray, LiquidBiopsy, Ephesia, HB-chip, CTC-Chip, GEM chip, GO chip, CTC-iChip, IsoFlux System, OncoCEE, IMS, BioFluidica Modular Sinusoidal Microsystem, BioFluidica Liquid Scan, MagRC, Novel cytosensor based on BSA@Ag@Ir MONs and Ocb40//AuNPs, rare-cell scWB (Milo), GILUPI CellCollector, Gilupi Nanodetector, ChimeraX-i120 Platform, online integrated inertial-magnetophoresis microfluidic chip-ICP-MS system, FACS, pluriBead, CellSieve, NYONE	High specificity, extended coverage by combining multiple markers, automation and clinical validation (Rzhevskiy et al., 2025)	Dependence on antigen expression, not applicable to non-epithelial tumors, issues of purity and false positives, influence of process complexity on the recovery rate.
	Immunocapture negative isolation and enrichment	EasySep, RosetteSep, QMS, CTC-iChip, MARS Bar, CTC-FIND, Dynabeads	Independent of specific surface markers, preservation of CTC integrity, applicable to heterogeneous tumors, wide clinical applicability.	Relatively low recovery rate, insufficient purity, omission of key cell subpopulations, technical complexity.
Other methods	Isolation and Enrichment Methods Combined with Nanotechnology	CellSearch, NanoVelcro, GO chip, MagRC, IMS, Novel cytosensor based on BSA@Ag@Ir MONs and Ocb40//AuNPs, PdPtCuRu MNS, Gold nanoparticles (AuNPs), Gilupi Nanodetector, TetherChip	High sensitivity and specificity (Loeian et al., 2019), flexibility and diversity (Ankeny et al., 2016), compatibility with downstream analysis.	Issues of repeatability and stability, challenges in clinical transformation, limitations and deviations (He et al., 2019), technical and operational complexity.

will be distributed in a specific layer of the gradient medium; while CTCs, which have a relatively low density, are usually enriched near the upper layer.

Charge/Electrical field force-based isolation and enrichment

By leveraging the differences in charge characteristics and responses to the electric field force between CTCs and other blood cells, CTC separation and enrichment can be achieved. Dielectrophoresis (DEP) technology generates a non-uniform electric field by applying a high-frequency alternating current to a microelectrode array. Relying on the disparities in the dielectric characteristics of cells, CTCs and normal blood cells exhibit distinct movement patterns within an electric field, thus enabling the enrichment of CTCs (Grüntkemeier et al., 2022).

Electrophoresis technology, on the other hand, takes advantage of the characteristic that charged particles move towards the opposite electrode in an electric field. Given the different surface charges of CTCs and blood cells, their migration speeds in the buffer solution vary. Separation is achieved by adjusting the electric field strength, electrophoresis time, and selecting an appropriate buffer solution (Ye et al., 2024). Electrostatic adsorption technology achieves the enrichment of CTCs by constructing a separation interface or material with a specific charge. For example, the surface of the microfluidic chip channel can be modified, or charged nanoparticles can be used. CTCs are adsorbed due to electrostatic attraction (Li et al., 2020).

Photoacoustic effect-based isolation and enrichment

When a biological sample containing CTCs is irradiated with short-pulse laser light, due to the differences in light absorption characteristics between CTCs and surrounding blood cells, different degrees of photothermal conversion will occur. Certain molecules

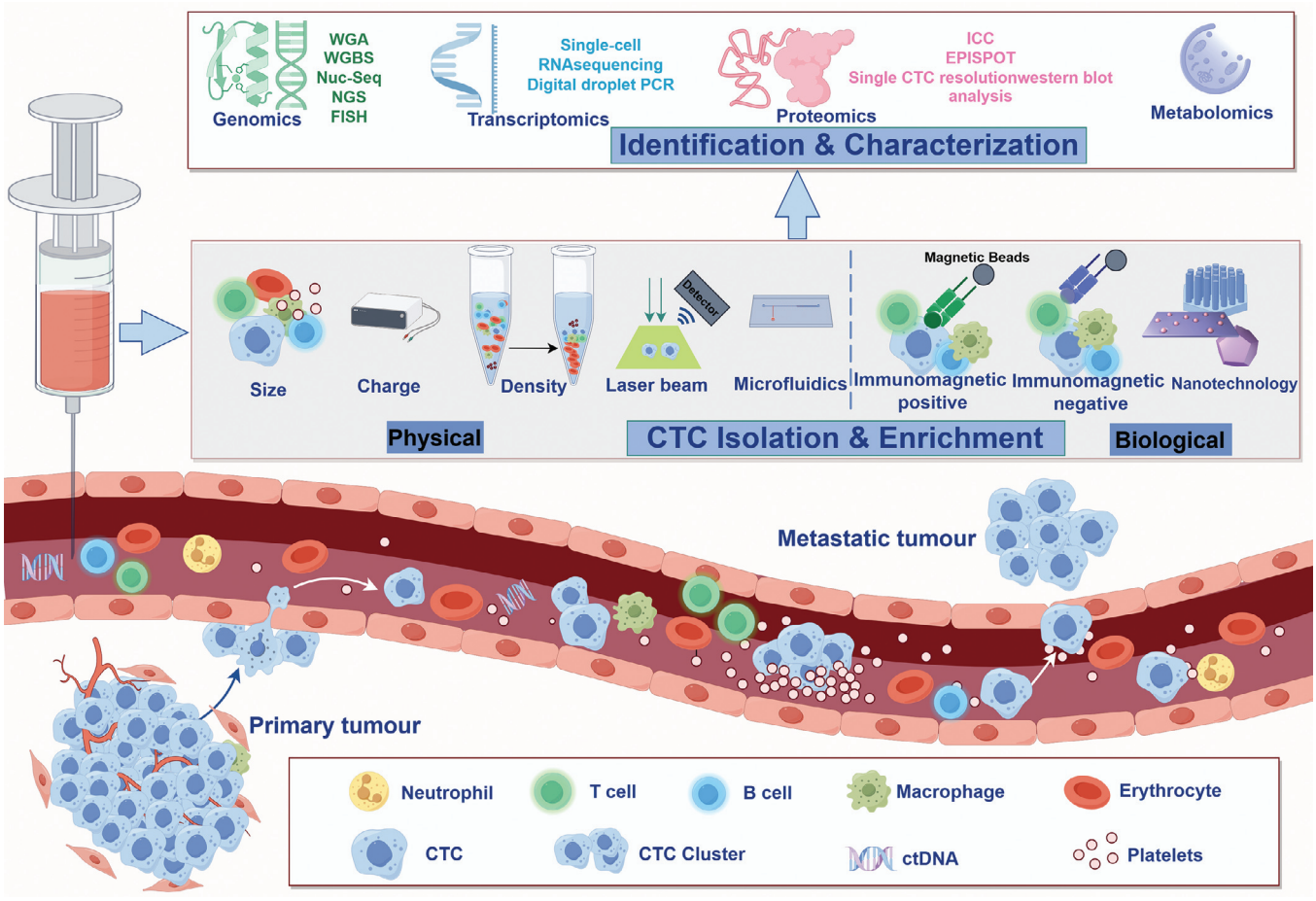

Fig. 1. CTC isolation, enrichment, and downstream analysis. After CTCs detach from the tumor and enter the bloodstream, by drawing peripheral blood, they can be isolated and enriched based on physical and biological characteristics using techniques such as size-based separation, charge-based separation, density-based separation, and immunomagnetic beads. Subsequently, downstream genomic, transcriptomic, proteomic, and metabolomic analyses can be carried out to identify and characterize CTCs. This figure was created in Figdraw.

or structures within CTCs may have a stronger absorption capacity for specific wavelengths of light. After absorbing light energy, it is rapidly converted into heat energy, which in turn causes local thermal expansion and generates photoacoustic signals (Pang et al., 2024). This photoacoustic signal differs from the signals generated by surrounding blood cells in characteristics such as amplitude and frequency. By using a highly sensitive photoacoustic detector, it is possible to accurately capture the unique photoacoustic signal produced by CTCs (Hai et al., 2020). Through the analysis and processing of these signals, the position of CTCs in the sample can be located. Subsequently, by combining microfluidic technology or other manipulation methods, CTCs can be separated and enriched from the complex environment of the blood sample.

Microfluidic-based isolation and enrichment

The microfluidic system is usually built on a microfluidic chip, which is equipped with a channel network of micrometer-scale dimensions (Luo and He, 2020). It operates on the principle of leveraging the variances in multiple physical and biological features of CTCs and other blood cells to realize precise isolation in a micro-sized fluid environment. For example, based on differences in cell size, microchannels or filtering structures of specific sizes are designed in the microfluidic chip. When a blood sample containing CTCs flows through these channels, CTCs with larger sizes will be retained or guided along a specific path, while smaller blood cells will pass through smoothly, thus preliminarily achieving the enrichment of CTCs (Au et al., 2017). In addition, by taking advantage of the laminar flow characteristics of the fluid in the microfluidic system and combining them with differences in cell deformability, CTCs can also be effectively separated. Normal blood cells, such as red blood cells, have good deformability and can change their shapes to adapt to the fluid flow and pass through narrow channels in laminar flow. However, CTCs have poor deformability and exhibit different flow behaviors under the same conditions, so the two can be distinguished accordingly.

Immunocapture methods

Immunomagnetic positive isolation and enrichment

The positive enrichment method using immunomagnetic beads has the advantage of high specificity. It is based on the specific binding between antigens and antibodies in immunology and uses magnetic beads to achieve precise separation and enrichment of CTCs. The specific operation is to conjugate antibodies against specific antigens on the surface of CTCs to the surface of magnetic beads. When a blood sample containing CTCs is mixed with immunomagnetic beads, the antibodies on the magnetic beads bind to the antigens on the surface of the CTCs. Under the action of a magnetic field, the magnetic beads carrying CTCs are separated from other blood cells, thus achieving the purpose of enrichment.

The CellSearch system is a typical example of this method and is the first CTC detection technology approved by the US Food and Drug Administration (FDA). It employs magnetic beads that are coated with antibodies targeting EpCAM to seize CTCs; this approach is frequently applied in the prognostic evaluation of metastatic breast cancer (Li et al., 2024), colorectal cancer (Cieslik et al., 2025), and prostate cancer (Cieślikowski et al., 2023). However, during the metastasis of cancer cells, EMT is likely to occur, leading to the downregulation or absence of the expression of epithelial markers such as EpCAM. As a result, CTCs that have undergone EMT are difficult to capture, resulting in missed detections (Xu et al., 2024). Moreover, non-epithelial cancers (such as melanoma and sarcoma) do not express EpCAM, making this method unable to detect CTCs derived from these tumor types.

To overcome the limitations of the CellSearch system, great research efforts have been devoted to the development of novel technologies. The MACS kit uses superparamagnetic microbeads coated with multiple antibodies (such as those against EpCAM, cytokeratin (CK), human epidermal growth factor receptor 2 (HER2), etc.) to isolate CTCs (Bartkowiak et al., 2025). After the whole blood sample is incubated with the microbeads, the separation of the CTC-microbead complexes is achieved in a magnetic field environment. The MagSweeper features a unique design. It uses a neodymium rod covered with a non-adhesive plastic sleeve to scan and capture CTCs in a whole-blood sample that has been incubated with magnetic beads coated with anti-EpCAM antibodies (Deng et al., 2014). This technology allows for multiple capture-release cycles, effectively enhancing capture efficiency. It can handle multiple samples simultaneously, and the captured CTCs remain viable after processing, facilitating subsequent analysis.

In prostate cancer research, the technology of using magnetic beads coated with antibodies against prostate-specific membrane antigen (PSMA) to capture CTCs has played an important role in disease monitoring and treatment plan formulation (Liu et al., 2024). In the context of breast cancer, a research team has employed magnetic beads coated with dual antibodies targeting both EpCAM and HER2. This approach has significantly enhanced the specificity and efficiency of CTC capture, enabling more accurate detection of CTCs in the blood of breast cancer patients (Nini et al., 2020). In the field of lung cancer, researchers have developed immunomagnetic beads targeting specific lung cancer-related antigens. For example, magnetic beads coated with antibodies against specific proteins on the surface of certain lung cancer cells can more precisely enrich lung cancer CTCs, providing strong support for the early diagnosis of lung cancer and the monitoring of treatment efficacy (Xie et al., 2025). Some studies have made use

of these immunomagnetic beads to carry out examinations on lung cancer patients. They discovered that these beads can efficiently identify CTCs in the blood of early-stage lung cancer patients, thus offering a foundation for early disease intervention.

In addition, some positive enrichment technologies using immunomagnetic beads are constantly emerging. For example, some research has combined nano-technology with immunomagnetic beads to prepare nano-immunomagnetic beads with special structures and functions (Li et al., 2018). These magnetic beads have a larger specific surface area, which enables them to couple with more antibodies, thereby enhancing the CTC-capture efficiency. Additionally, some research teams have developed an immunomagnetic bead enrichment technology based on microfluidic chips. By combining immunomagnetic beads with microfluidic chips, the enrichment of CTCs can be achieved within the channels of these chips (Kanayama et al., 2022). This technology can not only improve the enrichment efficiency but also reduce the sample volume required, enabling rapid and efficient detection of CTCs.

Immunomagnetic negative isolation and enrichment

The immunomagnetic negative-enrichment method utilizes anti-leukocyte antibodies bound to the surface of immunomagnetic beads to specifically remove the leukocyte population from the blood sample, thereby achieving the indirect enrichment of CTCs. In immunomagnetic negative-enrichment technology, the most commonly used method is to bind the anti-CD45 antibody to the magnetic beads. CD45 is a specific marker on the surface of white blood cells. In this way, under the action of a magnetic field, white blood cells will be adsorbed and separated by the magnetic beads, and the remaining cell population will be relatively enriched with CTCs (Lustberg et al., 2014). EasySep technology is based on this principle. It uses a magnetic field to retain white blood cells so that the final supernatant contains a population of CTCs that are not specifically labeled and are heterogeneous (Saini et al., 2025).

RosetteSep technology employs a more complex antibody combination. By using antibodies against multiple blood cell markers (such as CD2, CD16, CD19, CD36, CD38, CD45, CD66b, and glycophorin A), it forms a rosette network of "unwanted blood cells" after being mixed with the blood. Subsequently, through Ficoll density centrifugation, white and red blood cells in the sample can be removed. In addition, recent studies have found that there are some special interactions between CTCs and white blood cells, such as CTC-white blood cell clusters and "tumacrophages" (a type of hybrid cell formed by the fusion of macrophages and cancer cells). These cells may be of great significance in the process of tumor metastasis, but they will be removed during the immunomagnetic negative-enrichment process, which may lead to the omission of some key information about tumor cells (Amantini et al., 2019).

Isolation and enrichment methods combined with nanotechnology

These methods take advantage of the unique physical and chemical properties of nanomaterials, such as large specific surface area, high biocompatibility, and ease of functional modification (Yun et al., 2024). By combining nanotechnology with traditional enrichment methods, the CTC capture efficiency and detection sensitivity can be significantly improved. Nanomaterials have diverse applications in CTC enrichment, and immunomagnetic beads modified with nanoparticles are a relatively common form.

For example, gold nanoparticles have good biocompatibility and stability, which can enhance the binding ability between antibodies and magnetic beads and improve the capture efficiency of immunomagnetic beads for CTCs. A research team developed a gold nanoparticle-herringbone CTC chip (NP-HB CTC-Chip). By modifying gold nanoparticles on the surface of the herringbone chip and combining them with anti-EpCAM antibodies, it not only achieves efficient capture of CTCs but can also use the AuNP-thiol exchange reaction to simply release the captured CTCs by adding glutathione (GSH). Moreover, this process can maintain CTC viability and their molecular characteristics (Rushton et al., 2021).

Graphene oxide (GO) chips are an important advancement in CTC enrichment via nanotechnology. Thanks to GO's large specific surface area, these chips have a high antibody-carrying capacity and more CTC contact opportunities, constructing an efficient capture platform by combining functionalized GO nanosheets on flower-shaped gold-patterned surfaces with anti-EpCAM antibodies, with an average capture rate of up to 85% for cell lines with different EpCAM expression levels (over 65% for low-EpCAM-expressing PC3 cells) (Yoon et al., 2013). Furthermore, thermo-responsive GO chips can improve capture and release efficiency, enabling the recovery of high-viability cells for downstream analysis (Yoon et al., 2016). Meanwhile, silica nanoparticle (SiNP) platforms use 10-micrometer silica nanoparticles coated with anti-EpCAM antibodies to modify the chip substrate and form a 3D capture structure for enhanced topological interaction and cell capture efficiency. These achieved a capture rate of 84-91% in MCF7-spiked healthy blood experiments, with a capture yield increase of over 40% compared with traditional planar silicon-based platforms (Wang et al., 2009, Rushton et al., 2021).

In addition to immunomagnetic beads modified with nanoparticles, carbon nanotubes also demonstrate unique advantages in CTC enrichment. The nanotube-CTC chip takes advantage of the preferential adhesion characteristics of cancer cells to the surface of carbon nanotubes, enabling the capture of CTCs without the use of EpCAM antibodies. When detecting breast cancer patients, this chip can effectively reduce the number of contaminating white blood cells, achieving high-purity

capture of highly invasive CTCs. However, this method requires a pre-treatment step of red blood cell lysis, and the optimal cell adhesion time is 48 hours (Loeian et al., 2019).

Multi-parameter isolation and enrichment

Multi-parameter CTC sorting integrates multiple cell parameters, breaking through the limitations of single-parameter sorting. It can separate CTCs more precisely and efficiently, providing a more comprehensive and reliable sample for cancer research and clinical diagnosis. This technology fully takes into account the complexity and heterogeneity of CTCs and comprehensively utilizes various physical and biological cell characteristics, greatly improving the accuracy and efficiency of sorting.

In multi-parameter sorting, the method that combines physical and biological characteristics is widely applied. The combination of microfluidic technology and immunomagnetic beads is a typical example. For example, the CTC-iChip, a sophisticated microfluidic device, initially processes blood samples with immunomagnetic beads. Leveraging the features of cell-surface antigens, it conducts positive selection using EpCAM antibodies or negative selection with antibodies directed against leukocyte markers like CD45, CD16, and CD66b. After that, it employs the deterministic lateral displacement technique to separate nucleated cells from red blood cells and platelets. Next, via inertial focusing, the cells traverse the microfluidic channel almost one by one, and target cells are further concentrated under the influence of a magnetic field (Karabacak et al., 2014). This multi-step and multi-parameter sorting method significantly improves the capture efficiency and purity of CTCs. Moreover, it can handle relatively large-volume blood samples, enabling high-throughput detection. Experiments have shown that the CTC-iChip can achieve good sorting results, whether for cell lines with high EpCAM expression or cells in different EMT states (Ozkumur et al., 2013).

Another similar technology is the IsoFlux system, which also combines microfluidics and immuno-magnetic bead technology. After a whole-blood sample enters the system, it is first combined with antibody-coated magnetic beads for incubation and then guided through a microfluidic chip. In the "isolation zone", CTCs are captured using an external magnetic field. This system can process multiple samples in parallel and offers separation kits with various antibody combinations. For example, there are kits targeting EpCAM, EpCAM/EGFR, and those containing mesenchymal markers (EpCAM/EGFR/Vimentin/N-cadherin), which can comprehensively capture CTCs with different phenotypes, greatly enhancing the ability to detect CTC heterogeneity.

Multi-parameter sorting technologies that utilize the physical characteristics of cells are also continuously evolving. Some microfluidic chips achieve CTC sorting by comprehensively considering parameters such as cell size, deformability, and electrical properties. For example, the Parsortix device, based on microfluidic technology, has a chip with a gradient-changing separation structure. Given that CTCs are generally larger and less deformable than white blood cells, the device captures CTCs through gaps of specific sizes, allowing other smaller and more deformable blood cells to pass through (Saini et al., 2025). The ClearCell FX1 platform utilizes the principles of Dean flow and inertial focusing in a spiral microchannel. Based on the properties of cell size and deformability, it concentrates and maintains the larger CTCs on the inner portion of the spiral channel, while other blood cells move through the outer portion (Hou et al., 2013).

Moreover, multi-parameter sorting methods based on DEP technology have gradually attracted attention. DEP technology separates cells by utilizing their different dielectric properties in a non-uniform electric field. For example, ApoStream technology combines continuous field-flow assistance with DEP technology. It obtains peripheral blood mononuclear cells through the Ficoll-Paque gradient separation method and then separates CTCs in a microfluidic system based on differences in cell dielectric properties (O'Shannessy et al., 2016).

Sequencing and characterization of CTCs

CTC sequencing and characterization are crucial aspects in the field of cancer research, providing vital information for a profound understanding of the biological characteristics of tumors, disease progression, and the advancement of precision medicine. With the help of a series of advanced technologies, scientists can conduct a comprehensive and in-depth analysis of CTCs at multiple levels, including genes, transcripts, and proteins, thus revealing their heterogeneity and dynamic change patterns.

At the genetic level, whole-genome sequencing (WGS) and whole-exome sequencing (WES) play an indispensable role, providing a comprehensive and in-depth perspective for exploring the genetic characteristics of CTCs. Through WGS, researchers can conduct unbiased detection of the entire genome and discover various types of genetic variations, including single-nucleotide variants (SNVs), copy-number variants (CNVs), and chromosomal structural variations. For example, by sequencing single CTCs from lung cancer patients after whole-genome amplification, SNVs and insertions/deletions (INDELs) can be detected (Ni et al., 2013).

In prostate cancer research, WES technology revealed the CNV heterogeneity among patients and individual cells. At the patient level, there are obvious differences in the occurrence regions and frequencies of CNVs in prostate cancer CTCs from different patients. For example, in the CTCs of some patients, CNVs occur in the chromosomal region where the *androgen receptor*

(*AR*) gene is located, resulting in changes in the copy number of the *AR* gene, which in turn affects the androgen signaling pathway. This is closely related to the response and drug resistance of prostate cancer to androgen deprivation therapy. In the CTCs of some patients, CNVs exist in gene regions related to cell-cycle regulation and DNA damage repair, and these changes may affect the proliferation of tumor cells and their sensitivity to chemotherapeutic drugs. At the single-cell level, there is also CNV heterogeneity among different CTCs from the same patient. Even CTCs from the same primary tumor focus carry different CNVs. This single-cell CNV heterogeneity reflects the diversity of tumor cells during the evolution process and may be a mechanism for tumor cells to adapt to different microenvironments and evade treatment pressure. Some cells may gain a growth advantage through specific CNVs, thus playing an important role in the development and metastasis of tumors (Mishra et al., 2025).

In addition to analyzing the entire genome or exome, the precise detection of specific genes is of irreplaceable value in guiding clinical treatment. Take non-small cell lung cancer (NSCLC) as an example. The mutation status of the *EGFR* and *KRAS* genes is a crucial factor in determining whether to use targeted drugs such as EGFR tyrosine kinase inhibitors. Clinical studies have found that during the targeted treatment of some NSCLC patients, drug-resistant mutations, such as the T790M mutation, can occur in CTCs. The emergence of this mutation renders the originally effective targeted drugs ineffective, leading to tumor recurrence or progression. Therefore, real-time monitoring of the mutation status of these key genes in CTCs can help detect drug resistance in a timely manner, adjust treatment strategies promptly, and select more effective treatment options (Jan et al., 2018).

Transcriptome analysis zeroes in on the RNA aspect of CTCs. The principle of this technique involves first reverse-transcribing the mRNA in cells into cDNA, followed by amplification and sequencing to obtain a unique gene expression profile for each cell. In breast cancer research, single-cell transcriptome sequencing of CTCs has revealed significant heterogeneity in the states related to EMT and MET (mesenchymal-epithelial transition) among CTCs within patients. Some CTCs exhibit a stem-cell-like phenotype. These CTCs with stem-cell characteristics possess stronger self-renewal and differentiation capabilities and are closely associated with tumor recurrence and metastasis (Cheng et al., 2019). In prostate cancer research, RNA sequencing technology has revealed that the non-canonical Wnt signaling pathway is activated in the CTCs of patients resistant to anti-androgen therapy. This discovery provides potential targets for the development of novel therapies targeting the drug-resistance mechanism of prostate cancer. It is hoped that by interfering with this signaling pathway, tumor drug resistance can be overcome, and the treatment efficacy can be improved (Lu et al., 2019).

Characterization at the protein level is of crucial significance for directly understanding the functional state of cells, as proteins are the direct executors of cellular functions. Imaging mass spectrometry technology enables the quantitative analysis of multiple proteins at the single-cell level. By binding the proteins in cells to specific antibodies and using a mass spectrometer to detect the labeled proteins, researchers can obtain the protein expression profile of single cells. This technology can accurately detect the expression levels of proteins and also provide information on their intracellular localization, which helps to gain an in-depth understanding of protein functions and interactions. The microfluidic-based western blot technology provides another powerful approach for single-cell protein analysis. This technology utilizes the tiny channels of microfluidic chips and highly sensitive detection methods to measure the expression of a group of proteins at a single-cell resolution. Through the analysis of these protein expression data, researchers can gain a profound understanding of the biological characteristics of CTCs, such as the processes of cell proliferation, differentiation, and apoptosis, as well as the roles of these processes in tumorigenesis and development (Sinkala et al., 2017).

In addition to the analysis at the gene and protein levels, other characteristics of CTCs have also attracted extensive attention. Morphological features, including cell size, shape, nucleoplasmic ratio, etc., although relatively intuitive, contain important information. Through microscopic imaging technology, researchers can conduct detailed observation and analysis of CTC morphology (Cohen et al., 2022). Research has found that the morphological changes of CTCs are closely related to the malignancy and metastatic potential of tumors. For example, some CTCs with irregular shapes and a relatively large nucleoplasmic ratio often have higher invasiveness and metastatic capabilities (Jesenko et al., 2021). In addition, the metabolic characteristics of CTCs have gradually become a research hotspot. There are significant differences in metabolic patterns between tumor and normal cells. By analyzing the metabolites of CTCs or the expression of genes related to metabolic pathways, it is possible to reveal the energy-metabolic mode of tumor cells, which provides ideas for the development of new treatment strategies. Research has found that the CTCs of certain cancers have unique metabolic characteristics. They may rely on specific metabolic pathways to maintain their survival and metastatic capabilities (Francescangeli et al., 2021). In response to these metabolic differences, researchers can design specific metabolic inhibitors to block the energy supply of tumor cells, thereby inhibiting tumor growth and metastasis.

Although the sequencing and characterization of CTCs face many challenges, such as the extremely low CTC content in blood, difficulties in obtaining sufficient high-quality CTCs for analysis, existing enrichment

technologies failing to meet high-sensitivity detection needs, complex separation and detection operations that can damage CTCs and affect accuracy, and variations in sensitivity, specificity, and repeatability among different technologies along with the lack of unified standards restricting clinical use. Yet, with technological progress, CTC sequencing and characterization still hold great promise in future cancer research and clinical practice. The deep integration of microfluidics, nanotechnology, and sequencing is expected to boost CTC enrichment and detection. These technological strides will lay a firmer foundation for precision cancer treatment and are anticipated to improve patients' treatment results and survival prospects.

Clinical applications of liquid biopsy of CTCs

CTC liquid biopsy is of great significance in the clinical management of cancer. A variety of CTC isolation and enrichment techniques have been applied to various types of tumors (Table 2). In terms of early diagnosis, it is expected to detect cancer when the tumor is still invisible. In terms of prognostic assessment, the number of CTCs, their molecular phenotypes, and the related cellular conditions can all provide a basis for judging the development of the disease. A large number of CTCs, specific phenotypes, or special related cells often indicate a poor prognosis. When monitoring the treatment response, changes in CTC number and alterations in their molecular characteristics can reflect the treatment effect and drug resistance, assisting doctors in adjusting treatment strategies in a timely manner. It can also guide personalized treatment by analyzing the genetic and molecular information of CTCs.

Early diagnosis of cancer

CTC-based liquid biopsy has the potential to detect cancer at an early stage when traditional imaging methods are not sensitive enough. Since CTCs may be present in the blood before the primary tumor can be detected by imaging, they can serve as early warning signals. For example, in breast cancer research, CTCs have been detected in a considerable proportion of early-stage patients. For instance, 41% of patients in stage T1 and 47% of patients with negative axillary lymph nodes have had CTCs detected (Thery et al., 2019). Despite advancements, the scarcity of CTCs and the sensitivity limitations of current techniques remain major obstacles. Currently, novel technologies grounded in microfluidics and nanotechnology are under development, with the objective of enhancing the sensitivity of CTC detection for the early diagnosis of cancer.

Prognostic assessment

The presence and quantity of CTCs in the blood are closely related to cancer prognosis. In many types of cancers, including breast cancer, prostate cancer, lung cancer, and colorectal cancer, a higher number of CTCs is often associated with a poorer prognosis. In the case of metastatic breast cancer, the CellSearch system, which is authorized by the FDA to count CTCs, has revealed that patients with a higher CTC count ($\geq$ 5 CTCs per 7.5 milliliters of blood) have significantly shorter overall survival and progression-free survival (Hattori, 2025). In addition, CTC molecular phenotypes, such as the expression of EMT and stemness-related markers, also have prognostic significance (Su et al., 2020). CTCs with a mesenchymal phenotype or high expression of stemness markers are generally associated with a higher metastatic potential and a poorer prognosis for patients. Analyses of cells associated with CTCs, such as platelets, macrophages, and cancer-associated fibroblasts, can also provide valuable prognostic information. For example, the presence of CTC-platelet clusters is linked to a higher risk of metastasis.

Monitoring treatment response

During the treatment process, a decrease in the number of CTCs is generally associated with a favorable treatment response, while an increase may indicate treatment resistance or disease progression. During chemotherapy for metastatic breast cancer, patients who show a good response to the treatment generally witness a decrease in CTC counts once the treatment commences. In addition, changes in the molecular characteristics of CTCs during treatment can provide clues about the development of drug resistance. For example, in patients with EGFR-mutated NSCLC who are receiving tyrosine kinase inhibitor treatment, drug-resistant mutations (such as the T790M mutation) that occur in CTCs can be detected through CTC analysis (Maheswaran et al., 2008). This information enables clinicians to adjust treatment strategies in a timely manner, which has the potential to improve treatment outcomes for patients.

Guiding personalized treatment

CTCs can serve as a source of tumor-specific genetic and molecular information, which is crucial for guiding personalized treatment. By analyzing CTC gene mutations, gene expression profiles, and protein markers, the most appropriate treatment method can be selected for individual patients. For example, in prostate cancer, detecting the AR splice variant V7 (AR-V7) in CTCs can help predict the response to androgen deprivation therapy. Patients with AR-V7 positive CTCs are more likely to develop resistance to certain drugs, and this information can be used to switch to alternative treatment regimens, such as taxane chemotherapy (Armstrong et al., 2020). In addition, the *in vitro* culture of CTCs can be used to test the sensitivity of tumor cells to different drugs, providing personalized drug

Table 2. Clinical applications of various CTC isolation and enrichment techniques.

Techniques	Underlying principle	Description	Tumor types applied to
Ficoll-Paque	Density	Separates cells by density gradient centrifugation (Bartkowiak et al., 2025).	LIHC, STAD, BRCA, COADREAD, PRAD
OncoQuick	Density	Enriched CTCs between barrier & medium (Barber et al., 2024).	LIHC, GBM, COADREAD, BRCA, SKCM, OV, CESC, UCEC
RareCyte (AccuCyte - CyteFinder)	Density	Isolates CTCs via density gradient separation followed by staining and microscopy detection (Malkawi et al., 2023).	COADREAD, PRAD, BRCA, BLCA
RosetteSep	Density, Immunocapture negative	Depletes unwanted blood cells using density centrifugation combined with antibody complexes (Aggarwal et al., 2024).	LIHC LUNG, BRCA, MCC, PDAC, HNSC
Circulogix FaCTChecker	Size/deformability	Automatic filtration-based CTC enrichment system (Lin et al., 2010).	-
Vortex Biosciences VTX-1	Size/deformability	Laminar microvortices to isolate and concentrate CTCs (Liu et al., 2024).	LUNG, BRCA, UVM, PRAD
ScreenCell	Size/deformability	Size exclusion technology for isolating rare circulating cells (Barber et al., 2024).	GBM, BRCA, HNSC, PRAD, BLCA, SKCM, COADREAD, ESCA, LUNG
ISET	Size/deformability	Enriches CTCs based on their larger size compared with blood cells (Qi et al., 2024).	PRAD, BRCA, Desmoid tumor, COADREAD, OV, LUNG, KIRC, diffuse glioma, UVM, CHOL, LIHC, OSCC, READ, COAD, GIST
MCA	Size/deformability	Based on size and deformability, cells are enriched through closed/open chip methods (Cohen et al., 2023).	LUNG, LIHC
CellSieve	Size/deformability, Immunocapture positive	A 7-μm membrane short-time filtration tech enriches CTC counts by antibody staining, enriches CK-neg CTC clusters with malignancy evaluation limits (Reduzzi et al., 2021).	BRCA
CTC-FIND	Size/deformability, Immunocapture negative, Charge/electrical	Enrich CTCs via size, immunomagnetic negative selection & dielectrophoretic enrichment (Kozuka et al., 2021).	PRAD, BLCA, KIRC, PAAD, COADREAD
ANGLE Parsortix	Size/deformability, Microfluidics	Microfluidic cassette technology captures CTCs by non-deformability and large size (Saini et al., 2025).	LUNG, PRAD, BRCA, COADREAD, HNSC, OV, CUPs, ESCA, RCC, SARC
ClearCell	Size/deformability, Microfluidics	CTCs are separated by size in a spiral microchannel via inherent centrifugal forces (Martel et al., 2023).	UVM, BRCA, LUNG
FAST	Size/deformability, Microfluidics	A centrifugal microfluidic platform separates CTCs from whole blood by size via porous membrane and counts via immunofluorescence microscopy (Dong et al., 2020).	PRAD, OV, LUNG, COADREAD, ESCC, STAD, BRCA
A customized DLA biochip	Size/deformability, Microfluidics	Spiral-microchannel-based microfluidic chip customized for DLA product processing (Guglielmi et al., 2020).	-
FMSA	Size/deformability, Microfluidics	Device performance in capture efficiency, leukocyte enrichment, viability and proliferability was characterized (Kaifi et al., 2015).	COADREAD, BRCA, LUNG
GEDI chip	Size/deformability, Microfluidics	Chip combines 3D structure with anti-PSMA antibodies (Kirby et al., 2012).	PRAD
GEM chip	Size/deformability, Microfluidics	High-performance microchip based on optimized micromixer structures enhances flow to maximize CTC-antibody surface interaction (Farago et al., 2019).	PAAD, LUNG
DLD	Size/deformability, Microfluidics	A pillar-array-based passive microfluidic method separates cells by size (Tang et al., 2022).	LIHC
CFD	Size/deformability, Microfluidics	Integrates filtration into DLD for cell sorting, achieving high-throughput and clog-free separation via cascaded microfluidics (Liu et al., 2021).	LUNG
CTC-iChip	Size/deformability, Microfluidics, Immunocapture positive, Immunocapture negative	Combines microfluidics, inertial focusing, and immunomagnetic selection for CTC isolation (Guo et al., 2023).	PRAD, BRCA, LIHC, UVM, SKCM, PDAC, LUNG
Cytophone	Immunocapture positive	Detect CTCs by laser, ultrasound in photoacoustic flow cytometry (Galanzha et al., 2019).	SKCM
Puncher	-	Technology combines silicon chips, fluorescence imaging, & punching methods (Pereira-Veiga et al., 2024).	BRCA
LCM	-	A laser-based tech precisely isolates specific cells from complex populations, keeping cells intact for analysis (Zhao et al., 2021).	LUNG PRAD,
FACS	Charge/electrical, Immunocapture positive	A flow cytometry-based tech sorts specific cells in mixed populations by cell fluorescence labeling (Chauhan et al., 2024).	STAD, COADREAD, OSCC, BRCA, BLCA, PRAD, PDAC, PAAD, LUNG, LIHC
DEPArray	Charge/electrical, Microfluidics, Immunocapture positive	An automatic image-based sorter for isolation of pure CTCs (Di Trapani et al., 2018).	BRCA, CUPs, ESCA, RCC, SARC, EC, OV, SKCM, STAD, LUNG

Table 2. (Continued).

ApoCell ApoStream	Charge/electrical, Microfluidics	Uses DEP field-flow assist to separate non-hematopoietic cells from PBMCs by exposing laminar-flow cells to a critical AC frequency (Balasubramanian et al., 2017).	BRCA, LUNG, OV
CellMag	Immunocapture positive	Based on immunomagnetic beads, it captures epithelial cells via anti-EPRADAM antibodies to enrich CTCs (Saini et al., 2024).	LUNG
GILUPI CellCollector	Immunocapture positive	Overcomes blood volume limits of other methods to isolate CTCs from *in vivo* peripheral blood (Gorges et al., 2016).	PRAD, BRCA PRAD, KIRC, LUNG, COADREAD, HNSC
Gilupi Nanodetector	Immunocapture positive, Nanotechnology	Insert nanodetector/wire into patient's vein and dock cells on antibody-laden “nano polymer threads”.	-
NYONE	Immunocapture positive	Epithelial marker-based immunofluorescence staining and automated microscopy detection (Hendricks et al., 2021).	COADREAD
EPISPOT	Immunocapture positive	Detects CTC-secreted proteins with capture and detection antibodies (Budna-Tukan et al., 2019).	PRAD, COADREAD, HNSC, BRCA
MACS	Immunocapture positive	Under an external magnetic field, magnetic bead-labeled target cells are adsorbed for separation from other cells (Bartkowiak et al., 2025).	BRCA, LIHC, SKCM, EC, PAAD, LUNG, COADREAD, KIRC
MagWIRE	Immunocapture positive, Nanotechnology, Microfluidics	Uses flexible magnetic wire to capture *in vivo* CTCs. It needs pre-injection of EPRADAM-coated magnetic particles, however, long-term use may be limited by iron-overload risk.	-
MagSweeper	Immunocapture positive	CTCs are captured with magnetic rods, which are covered by removable plastic sleeves (Sieuwerts and Jeffrey, 2012).	BRCA, PRAD, BRCA
IVFC	Immunocapture positive	Enables real-time CTC detection without blood collection (Wei et al., 2017).	BRCA, PDAC
FCM	-	Passes suspended cells/particles through the detection area individually, detects laser-induced signals for cell analysis (Alsayed et al., 2021).	HNSC, COADREAD, BRCA
Imaging Flow Cytometry (ImageStream)	-	A 12-channel tech detects fluorescently labeled cells, evaluating 12 markers and obtaining micro-images for multi-parameter phenotyping (Ruiz-Rodríguez et al., 2021).	COADREAD
Attune NxT	-	Fluorescently labels cells and uses flow cytometry to detect target cells in a sample (Gruijs et al., 2022).	BRCA
MI-FCM	-	Combines traditional flow cytometry's high-throughput cell analysis with imaging technology's cell morphology observation (Takahashi et al., 2025).	COADREAD
AdnaTest	Immunocapture positive	Enriches CTCs with magnetic beads conjugated to antibodies targeting epithelial markers (Liu et al., 2024).	PRAD, OV, BRCA, COADREAD
ChimeraX-i120 Platform	Immunocapture negative, Immunocapture positive, Density	An automated platform integrating negative enrichment, immunofluorescent labeling, and ML-based identification (Wang et al., 2021).	LIHC, CHOL, BRCA, COADREAD, LUNG
PluriBead	Immunocapture positive	Captures CTCs using non-magnetic beads conjugated with monoclonal antibodies (Barber et al., 2024).	GBM
IMS	Immunocapture positive, Nanotechnology	Uses immunomagnetic beads to bind target cells and separate them by magnetic field (Gribko et al., 2021).	HNSC, LUNG
Cellsearch	Immunocapture positive, Nanotechnology	An immunomagnetic method for CTC enumeration using anti-EpCAM antibodies (Cieslik et al., 2025).	PRAD, GBM, COADREAD, LUNG, BRCA, MCC, PDAC, PAAD, HNSC, UVM, RCC, CUPs, ESCA, BLCA, HNSC, UCEC, LIHC, KIRC, STAD, NETs, BLCA, OV, SKCM
Microfluidic western blot	Microfluidics, Immunocapture positive	Performs single-cell protein analysis through microfluidic channels for high-resolution detection (Sinkala et al., 2017).	BRCA
CTC-μChip	Microfluidics, Immunocapture positive	A microfluidic device based on the principle of lateral magnetophoresis (Cho et al., 2020).	PRAD, BRCA
CTC-Chip	Microfluidics, Immunocapture positive	Based on microfluidics, it captures CTCs in a microfluidic chip chamber by attaching antibodies to micropillar surfaces (Kanayama et al., 2022).	LUNG, NPRAD, MPM, COADREAD, PRAD, LIHC, BRCA, ESCA
HB-Chip	Microfluidics, Immunocapture positive	A microvortex mixer ensures cell antibody-coated surface contact for CTC capture (Wang et al., 2016).	PRAD
NanoVelcro	Microfluidics, Immunocapture positive	Captures agents and embedded nanosubstrates enhancing CTC-surface capture affinity (Jiang et al., 2015).	PRAD, LUNG
NanoVelcro CTC chip	Nanotechnology, Microfluidics, Immunocapture positive	Anti-EPRADAM-coated nano-substrate combined with a microfluidic chaotic mixer boosts CTC capture and identification efficiency (Lee et al., 2022).	PDAC, PRAD, TSCC, NETs, LIHC, LUNG, STAD, PAAD, KIRC
IsoFlux System	Microfluidics, Immunocapture positive	Integrates microfluidics and immunomagnetic beads to isolate CTCs from whole blood (Espejo-Cruz et al., 2025).	LIHC, BLCA, BLCA, PDAC, COADREAD, PSCC, PAAD, ESCA, OV, PRAD

sensitivity tests for patients.

Conclusion

In recent years, significant progress has been made in CTC research, with major breakthroughs in aspects such as detection, enrichment, genomic sequencing, and clinical applications. These advancements have deepened our understanding of cancer metastasis and opened up new avenues for the diagnosis, prognostic

Table 2. (Continued).

OncoCEE	Microfluidics, Immunocapture positive	Captures CTCs in microchannels with antibody cocktails for enhanced specificity (Kalinsky et al., 2015).	BRCA
BioFluidica Modular Sinusoidal Microsystem	Microfluidics, Immunocapture positive	Antibody-coated sinusoidal channels isolate specific CTCs from whole blood.	-
Ephesia	Microfluidics, Immunocapture positive	It combines microfluidics and immunomagnetic sorting, with the core of using superparamagnetic bead self-assembly under a magnetic field (Autebert et al., 2015).	PRAD, BRCA
LiquidBiopsy	Microfluidics, Immunocapture positive	Uses flow-chip technology to isolate and analyze rare cells, including CTCs (Kobayashi et al., 2022).	BLCA, BRCA, LUNG, SKCM
Rare-cell scWB (Milo)	Microfluidics, Immunocapture positive	Based on traditional protein immunoblotting and improved by microfluidic technology (Hughes and Herr, 2012; Kang et al., 2016).	BRCA, GBM
Online integrated inertial-magnetophoresis microfluidic chip-ICP-MS system	Microfluidics, Immunocapture positive	An integrated inertial-magnetophoresis microfluidic chip online-coupled with ICP-MS for rapid CTC separation and precise detection (Cai et al., 2024).	BRCA
GO chip	Microfluidics, Immunocapture positive, Nanotechnology	Gold nanoparticles covered with graphene oxide nanosheets contribute to the growth of molecular chains designed to capture CTCs (Niu et al., 2024).	PRAD, BLCA
Nanotube-CTC-chip	Nanotechnology	Achieves cell capture via CTCs' preferential adhesion to carbon nanotube surfaces (Loeian et al., 2019).	BRCA
MagRC	Microfluidics, Immunocapture positive, Nanotechnology	Sorts CTCs based on magnetic intensity by microfluidics and magnetic labeling (Poudineh et al., 2017).	PRAD
TetherChip	Microfluidics, Nanotechnology	Microfluidic tech on a chip builds a nanosurface for non-adherent tumor cell capture, suspension, microtentacle fix, & CTC isolation in clinical analysis (Liu et al., 2025).	BRCA
Flow enrichment target capture Halbach (FETCH) magnetic separation system	Microfluidics, Nanotechnology	A Halbach-array device separates magnetic samples in continuous flow and enhances low-EPRADAM CTC capture (Liu et al., 2025).	PRAD, BLCA, LUNG
QMS	Immunocapture negative,	Performs negative selection of CTCs by depleting leukocytes with immunomagnetic beads (Tong et al., 2007).	HNSC
Dynabeads	Immunocapture negative, Immunocapture positive	A cell separation technique based on immunomagnetic beads (Drucker et al., 2020).	BRCA, PAAD, BLCA, KIRC, BLCA
EasySep	Immunocapture negative	Based on immunomagnetic bead negative selection, uses magnetic beads to bind and remove unwanted cells from the sample (Saini et al., 2025).	LUNG, BRCA, OSCC, COADREAD, CHOL
MagPure chip	Immunocapture negative	An immunomagnetic-based MagPure microfluidic chip depletes WBRCAs for CTC negative selection and provides purified samples for analysis (Descamps et al., 2022).	LUNG, BRCA
Epic Sciences	-	Collects peripheral blood, centrifuges, immunofluorescent-stains, scans, and uses the BRIA algorithm to identify candidate CTCs (Ruiz-Vico et al., 2025).	PRAD, BRCA
AuNPs	Nanotechnology	An effective platform for multivalent DNA aptamer assembly for efficient cell capture.	-
Novel cytosensor based on BSA@Ag@Ir MONs and Ocb40//AuNPs	Nanotechnology	A dual-recognition & BSA@Ag@Ir-based tech using Ocb40//AuNPs-modified electrodes detects CTCs via current signals sensitively and specifically (Shen et al., 2021).	-
PdPtCuRu MNS	Nanotechnology	A dual recognition-based tech uses KB & CeMOF-Au-modified electrodes and PdPtCuRu nanospheres to build an electrochem biosensor for CTC detection (Zhou et al., 2023).	-

LIHC: Liver hepatocellular carcinoma; BRCA: Breast invasive carcinoma; STAD: Stomach adenocarcinoma; COADREAD: Colon adenocarcinoma and rectum Adenocarcinoma; PRAD: Prostate adenocarcinoma; GBM: Glioblastomas; CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; UCEC: Uterine corpus endometrial carcinoma; UVM: Uveal melanoma; BLCA: Urothelial carcinoma; SKCM: Skin cutaneous melanoma; ESCA: Esophageal adenocarcinoma; KIRC: Renal clear cell carcinoma; READ: Rectal cancer; COAD: Colon cancer; CHOL: Intrahepatic cholangiocarcinoma; PAAD: Pancreatic cancer; NPRAD: Nasopharyngeal carcinoma; NETs: Neuroendocrine tumors; MPM: Malignant pleural mesothelioma; TSCC: Tongue squamous cell carcinoma; PSCC: Penile squamous cell carcinoma; SARC: Sarcoma

assessment, and treatment of cancer.

In the field of CTC detection and enrichment, numerous technologies have been developed, each with its own unique advantages and limitations. Immunocapture methods, such as the CellSearch system, are widely used. However, due to EMT, they face challenges in detecting CTCs with low EpCAM expression. Physical property-based methods, such as membrane filtration and microfluidic technology, provide label-free alternatives but have problems such as clogging and low purity. Nanotechnology-based methods show great promise in improving capture efficiency and sensitivity, but further optimization is still needed for their widespread clinical application. The continuous innovation of these technologies, including the development of combined methods, is expected to overcome the current limitations and enhance the level of CTC isolation and detection.

The genomic sequencing of CTCs provides valuable information for understanding tumor heterogeneity and evolution. Single-cell sequencing technology enables researchers to reveal genetic and transcriptional variations at the single-cell level and identify key drivers of cancer progression and drug resistance. However, challenges such as the low content of genetic material in CTCs and the need for more sensitive and accurate sequencing methods still remain to be addressed.

CTCs have great potential for clinical applications. As liquid biopsy biomarkers, CTCs can be used for the early diagnosis of cancer. In terms of prognostic assessment, the number of CTCs and their molecular phenotypes are related to the prognosis of patients with various cancers. Regarding treatment monitoring, alterations in the quantity and traits of CTCs throughout the treatment process are able to signal the treatment's effectiveness and the emergence of drug resistance. Moreover, CTCs can direct personalized treatment strategies by furnishing details about tumor-specific gene mutations and drug sensitivity patterns. However, to fully realize the clinical application value of CTCs, efforts in large-scale clinical validation, the establishment of standardized procedures, and cost reduction are crucial.

In conclusion, although significant progress has been made in CTC research, there are still obstacles to be overcome. Future research should focus on developing more sensitive and specific detection and enrichment methods, improving the genomic sequencing technology of CTCs, and establishing standardized guidelines for clinical applications. By addressing these challenges, CTCs have the potential to become an important part of precision medicine, enabling earlier cancer detection, more accurate prognostic prediction, and personalized treatment strategies, ultimately improving the treatment outcomes for patients.

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