Circulating Tumor Cells: A Review of Present Methods and the Need to Identify Heterogeneous Phenotypes

Lori M. Millner, Mark W. Linder, and Roland Valdes, Jr.
Department of Pathology and Laboratory Medicine, School of Medicine, University of Louisville, Louisville, KY, USA

Abstract. The measurement and characterization of circulating tumor cells (CTCs) hold promise for advancing personalized therapeutics. CTCs are the precursor to metastatic cancer and thus have the potential to radically alter patient treatment and outcome. Currently, clinical information provided by the enumeration of CTCs is limited to predicting clinical outcome. Other areas of interest in advancing the practice of pathology include: using CTCs for early detection of potential metastasis, determining and monitoring the efficacy of individualized treatment regimens, and predicting site-specific metastasis. Important hurdles to overcome in obtaining this type of clinical information involve present limitations in defining, detecting, and isolating CTCs. Currently, CTCs are detected using epithelial markers. The definition of what distinguishes a CTC should be expanded to include CTCs with heterogeneous phenotypes, and markers should be identified to enable a more comprehensive capture. Additionally, most methods available for detecting CTCs do not capture functionally viable CTCs. Retaining functional viability would provide a significant advantage in characterizing CTC-subtypes that may predict the site of metastatic invasion and thus assist in selecting effective treatment regimens. In this review we describe areas of clinical interest followed by a summary of current circulating cell-separation technologies and present limitations. Lastly, we provide insight into what is required to overcome these limitations as they relate to applications in advancing the practice of pathology and laboratory medicine.

Introduction

Metastatic disease is responsible for 90% of cancer related deaths [1]. The metastatic process is accomplished by a circulating tumor cell (CTC) successfully carrying out a series of processes. First, the CTC must detach from the primary tumor and intravasate into the blood stream. Once in circulation, the CTC must evade immune detection and extravasate into microvessels of a target tissue [2]. The successful formation of a metastatic lesion is dependent upon the CTC’s ability to adapt, survive, and induce neoangiogenesis in the target tissue [3]. CTCs have been identified in many cancers, including bladder [4], gastric [5], prostate [6], lung [7], breast [8], and colon cancer [9].

CTC detection is accomplished based on physical properties of the cell, including density and size as well as immune and cell-surface electrical properties. The Food and Drug Administration (FDA) has approved a method for CTC enumeration used to predict cancer patient outcome. The CellSearch™ by Veridex has been cleared for clinical use in breast [8], colorectal [10], and prostate cancer patients [11]. This method enriches cells using a magnetic ferrofluid containing antibodies against epithelial cell adhesion molecule (EpCAM). Cells are then stained for expression of cytokeratin (CK) 8, 18, and 19, all of which are intracellular structural proteins found in epithelial cells. The presence of these rare cells detected by CellSearch has been implicated in worse cancer prognosis and outcome [8]. Patients with levels of CTCs equal to or higher than 5 per 7.5 ml of whole blood, as compared with the group with fewer than 5 CTCs per 7.5 ml, had a shorter median progression-free survival (2.7 months vs. 7.0 months, \( p < 0.001 \)) and shorter overall survival (10.1 months vs. >18 months, \( p < 0.001 \)) [8]. Like the CellSearch method, the majority of CTC detection techniques rely on EpCAM and CK expression.
Lack of detection in breast cancer cell lines with low EpCAM expression. The percentage of CTCs not detected by the FDA-cleared CellSearch is unknown. However, a study examining EpCAM expression in 50 breast cancer cell lines indicated that 20% of cell lines had low levels of EpCAM expression [12]. Interestingly, these cells were also reported to have a basal-like phenotype with high expression of vimentin (a mesenchymal marker) and low expression of other epithelial markers, including CKs 8, 18, 19 and E-cadherin. This indicates that these aggressive, basal-like cell types would likely not be captured using the EpCAM-dependent CellSearch. These cell lines may be representative of CTCs that have experienced EMT, no longer express epithelial markers, and are likely not detected using CellSearch. Additionally, the study also examined EpCAM mRNA expression from CTCs collected from cancer patients and found that there was a subset of CTCs that had low expression of EpCAM. This suggests that CTCs expressing a mesenchymal phenotype would be difficult to capture. Because research indicates that tumor cells which have experienced EMT are more capable of causing metastasis, it is important that the definition of a CTC be expanded and markers identified to facilitate the capture of these formidable, metastasis-forming cells [12,13].

Progress in individualized cancer therapy. Much progress has been made in guiding individual therapy toward molecular characterization of the primary tumor. However, following development of metastatic disease, the treatment often continues to be based on molecular characterization of the primary tumor despite discordance between the primary and metastatic lesions [14]. Performing a biopsy on metastatic lesions is often impossible due to anatomical position or the presence of multiple metastatic foci. There is great potential for CTCs to be used as an alternative to tissue biopsy, providing a much less invasive method of monitoring molecular profile status. Clinical applications utilizing CTCs being explored include determination of treatment eligibility independent of primary tumor, real-time indication of treatment efficacy, organ-specific prediction of metastasis, and early detection as a liquid biopsy.

CTC Subtypes. It is of interest to identify subtypes of CTCs based on extracellular protein expression with associated metastatic potentials. Identifying a subtyping scheme for CTCs with known metastatic potentials would provide clinicians a more complete picture of their patient’s risk for metastasis. Understanding the danger posed by an individual’s CTCs would allow physicians to provide the most appropriate and personalized treatments for each patient.

Use of CTCs to determine treatment eligibility. CTCs are of interest in helping to predict the most effective and individualized treatment regimen for cancer patients. CTCs have been shown to possess genetic characteristics different than those of the primary tumor. Targeting the CTCs in addition to the primary tumor would allow some patients to receive treatment for which they would otherwise be ineligible. For example, a recent phase II trial was designed to evaluate the activity of lapatinib in metastatic breast cancer patients with HER2-negative primary tumors and HER2-positive circulating tumor cells (CTCs). Patients with HER2-negative primary tumors and HER2-positive CTCs received lapatinib treatment [15]. Although this study reported that only 1 out of 7 patients responded to treatment, this method to determine treatment eligibility based on CTC targets should be further examined.

Another clinical trial that is currently enrolling (clinical trial identifier: NCT01048918, sponsored by the City of Hope Cancer Center) is studying the characterization of CTCs to direct pre-operative and systemic therapy in breast cancer patients. The goal is to tailor treatment regimens based on expression of CTCs to better individualize therapy to the specific needs of a patient.

CTCs in real-time monitoring of treatment. An ongoing phase III clinical trial, SWOG SO500, is monitoring the effect of early changes in therapy in patients with high CTC counts based on the CellSearch method. Patients with 5 or more cells per 7.5 milliliters of blood following 3 weeks of treatment will be randomly assigned to either stay on their current chemotherapy regimen or switch to a different regimen. Patients with fewer than 5 CTCs will remain on the current treatment. Clinicians believe that switching treatments based on CTC status instead of relying on traditional clinical signs of progression may provide an opportunity for patients to receive more beneficial treatment.

CTCs to predict site of future metastatic lesion. There is interest in using CTCs to predict sites of future metastasis. Detection and knowledge of the molecular architecture of these cells may serve as biomarkers to signal metastasis-capable disseminating cells and indicate potential target organs [16]. Studies have not been completed in this area but it has been mentioned as an area of keen interest.

CTCs in early detection. An on-going clinical trial sponsored at the Walter Reed Army Medical Center is directed toward early detection of undetectable cancers by screening for CTCs (Trial ID: NCT01322750). There is evidence to suggest that the systemic spread of
cancer occurs early in the natural history of breast cancer and that current screening methods are unable to detect this early spread [17,18]. The goal of the study is to be able to identify subclinical disease with a simple blood test.

There are many clinical interests and potential uses for CTCs to improve patient outcome. However, because significant limitations exist in the ability to capture heterogeneous CTCs and to perform downstream functional analysis, these clinical interests will not be fully realized until these obstacles are overcome. There are two main hurdles to clear in the field of CTC detection and capture. The first is that because CTC detection relies on epithelial markers [19,20], populations of CTCs with a non-epithelial phenotype are not currently detected [21]. The second challenge regarding CTC detection and capture is that most CTC capture methods require permeabilization and do not allow the cell to remain viable. CTC viability is required to perform downstream functional analysis that would enable metastatic potential and other functional cell characteristics to be examined. Here we provide a summary of techniques used to capture CTCs, including various enrichment techniques based on cell density, negative depletion, magnetic activated cell sorting, size separation, and magnetophoretic activity. The detection methods that will be described include FACS, FAST, EpiSpot, CTC-Chip, and FISH, as well as their limitations.

**Enrichment**

Circulating tumor cells are very rare. CTCs account for 1 or fewer cells in $10^5 - 10^6$ peripheral blood mononuclear cells [22]. Typically, between 1 and 10 CTCs / mL of whole blood are detected in patients with metastatic disease [23]. Due to their rarity, most techniques require an enrichment step prior to detection of CTCs. This enrichment step typically separates CTCs from unwanted hematopoietic cells and results in a fraction that is enriched for CTCs and ready for detection techniques to be carried out. Physical properties that allow rare cells to be separated include cell size, density, migratory properties, and protein expression.

**Density-based cell separation.** Density-based cell separation is based on differential migration according to differences in buoyant density. The use of Ficoll, an inert polysucrose, to isolate intact leukocytes from whole blood was first described in 1967 [24]. Oncoquick (Greiner Bio One, Frickenhausen, Germany) is a commercially-available density gradient centrifugation device that is marketed for tumor cell enrichment and has been used to enrich CTCs from whole blood samples [25]. Density gradient centrifugation is now used widely as an enrichment step prior to detection by another method. Its limitation is non-specific loss of desired cells.

**Negative enrichment.** Another enrichment method is based on negative enrichment, or the depletion of the majority of leukocytes and erythrocytes. One example of this is the RosetteSep (StemCell Technologies, Vancouver, BC, Canada), which employs a complex of antibodies targeted against hematopoietic cells and crosslinks unwanted cells in human whole blood to multiple red blood cells (RBCs), forming immunorosettes. The density of the unwanted cells is increased by the formation of immunorosettes and causes them to pellet along with the free RBCs when centrifuged over a buoyant density medium such as Ficoll-Paque. As with density-based cell separation, the limitation of this technique is non-specific cell loss.

**Magnetic activated cell sorting.** A widely used enrichment method is magnetic activated cell sorting (MACS). MACS relies on antibody-based capture with magnetically labeled antibodies. MACS can be used for negative or positive enrichment, depending on the antibodies used. CD45 can be used to deplete leukocytes, while EpCAM can be employed for positive enrichment. MACS is capable of high throughput capacity but is unable to differentiate between low and high expression levels of any desired target [26]. The FDA-cleared method of CTC enumeration uses magnetically labeled EpCAM antibodies to positively enrich for CTCs from whole blood. The MagSweeper is an automated immunomagnetic cell separator for isolating rare endothelial cells [27]. The MagSweeper uses a magnetic arm to collect cells labeled with anti-EpCAM antibodies. Because most MACS applications separate based on only one parameter (i.e., the presence or absence of magnetization), new magnetic separation techniques with multiple parameter selection capabilities have been developed [28-30]. One such multi-target magnetic activated cell sorting (MT-MACS) has been described; it relies on microfluidic technology and large magnetic field gradients to achieve simultaneous, spatially-addressable sorting of multiple cell types [31]. Isolation of CTCs based on MACS is limited to the targets chosen.

**Separation based on size.** In addition to differences in density and immunomagnetic enrichment, separation based on size and deformability has been achieved. For example, isolation by size of epithelial tumor cells (ISET) is a filtration-based approach used to isolate epithelial cells [32]. A micromachined device that separates cells based on size and deformability has been described for enrichment or isolation of rare cells from peripheral
blood. When mixed with whole blood, cells from eight different cancer cell lines were isolated [33]. A label-free microdevice that separates rare cells based on size and deformability has been described and has successfully isolated breast and colon cancer cells [34]. This microdevice was capable of isolating tumor cells with an efficiency of 80% [34]. Development of a parylene membrane microfilter device for single-stage capture and electrolysis of CTCs in human blood followed by genomic analysis has also been described [35]. However, size-based enrichment techniques are limited since cell size can vary greatly within single populations [36].

Separation based on Magnetophoretic Mobility. A highly advanced method of cell enrichment and separation is achieved by the Quadrupole Magnetic Cell Sorter (QMS, Ikonosys, New Albany, Indiana, USA). The QMS separates cells based on magnetophoretic mobility and has the capacity to sort cells at a speed of up to 10 million cells per second. Magnetophoretic mobility refers to the movement of cells labeled with magnetic nanoparticles in a magnetic field. Cells can be collected into low, medium, or high mobility fractions. The QMS achieved 99% recovery of CD34(+)CD45(+) cells from peripheral blood leukocytes and is being evaluated for use in clinical-scale depletion of T cells for allogeneic stem cell transplantation [37]. With a single-step immuno-magnetic labeling, the QMS produced a 57-fold enrichment and 77.8% recovery of rare cancer cells [38]. The limitation of magnetophoretic mobility is the targets that are chosen.

Following enrichment of the desired cell population, many different CTC detection techniques can be employed, including fluorescence-assisted cell separation (FACS), fiber-optic array scanning (FAST), fluorescence-assisted in situ hybridization (FISH), nucleic acid detection, and microfluidic-based separation. These techniques are summarized in Table 1.

Detection

Fluorescence assisted cell sorting (FACS). Following enrichment, rare cells can be detected and characterized based on cytometric or nucleic acid techniques. The most widely used cytometric technique used to separate rare cell populations relies on antibody-based capture. Fluorescence-assisted cell sorting (FACS) is the most commonly used method for separating a specific population of cells when high purity is required [39]. Physical properties including cell size (forward scatter) and internal complexity (side scatter) can be measured. Based on its expression of desired targets, each cell is electrically charged, allowing for the collection of distinct populations by an electrostatic detection system. The most advanced flow cytometers can accommodate a flow rate of up to 50,000 cells per second with multiple parameter detection. An excellent detailed review has been written on the methods and applications of flow cytometry [40]. FACS has many applications because many parameters can be simultaneously measured [41]. A limitation to FACS is throughput; because each cell must be sorted individually, the amount of cells that can be analyzed is limited. Additionally, once cells are sorted by FACS, the viability of certain cell types can be reduced due to flow sorting conditions [42].

Fiber-optic array scanning technology (FAST). To more efficiently analyze high numbers of immunofluorescently-labeled rare cells, the fiber-optic array scanning technology (FAST) has been developed. This technology uses a fiber optic array to capture and analyze rare cells in real-time. The fiber optic array consists of a network of optical fibers that can be individually addressed to collect fluorescence signals from individual cells. This allows for high throughput analysis of rare cells in a single sample. FAST technology has been used to detect and isolate rare circulating tumor cells from human blood, and has demonstrated high specificity and sensitivity. The technology is still in development, and further improvements are needed to increase its clinical utility.
technology (FAST) was developed. FAST cytometry allows the cytomorphology of the prospective rare cells to be readily examined at any time. FAST is a separation and isolation method, based on laser printing optics, that excites cells and records emission over an exceptionally large field of view. Because larger volumes of peripheral blood can be analyzed, no additional enrichment step is required for FAST cytometry, reducing the risk of cell loss [43,44]. This method has been used to detect CTCs in stage IV breast cancer patients [44].

**Nucleic-acid-based detection.** CTCs have been identified by reverse transcription quantitative polymerase chain reaction (RT-qPCR) analysis of expressed cell specific makers. Detection of RNA is likely an indicator of a live cell, because RNA disappears quickly following cell death. Several studies have reported RT-qPCR to have higher sensitivity than immunocytochemistry [45]. This approach has been applied to enriched CTC populations to detect cell specific markers. Specifically, MUC-1 has been examined in breast cancer patients [46], cytokeratin (CK) 19 and CK 20 in colorectal cancer patients [47], hTERT in gastric cancer patients [48], and alphafetoprotein (AFP) in patients with primary liver cancer [49]. However, the RT-qPCR approach has been reported to have a high frequency of false positives due to sample contamination and expression of target genes in normal cells [50]. In addition to high rates of false positives, another issue with RT-qPCR is that once RNA has been collected from a single cell, the cell is no longer viable and cannot be used for advanced analysis such as cell-based assays like cell migration, adhesion, or sensitivity assays.

**Fluorescence-assisted in situ hybridization.** Fluorescence-assisted in situ hybridization (FISH) is a method used to detect the presence or absence of specific DNA sequences on chromosomes using fluorescent probes. It is highly accurate and used to detect chromosomal fetal abnormalities in maternal peripheral blood [51]. Although it is highly accurate, FISH is labor-intensive, requires a high skill level, and can be highly subjective. To combat these obstacles, another method developed for rare cell identification and analysis based on optical imaging is the Ikoniscope® imaging system (Ikonisys, New Haven, Connecticut, USA) [52-54]. Reports indicate detection of one CTC per milliliter of blood is possible [54]. These methods are also limited as research tools because cells are no longer viable following detection.

**Microfluidic Devices.** Dielectrophoresis (DEP) refers to the movement of neutral polarizable particles induced by electric field gradients. Methods in stem cell separation using DEP have been reviewed [55]. Most cell sorting techniques based on DEP rely on the combination of electrical fields with other emerging technologies, such as microfluidic laminar flows and optical tweezers. However, the addition of such harsh methods often reduces cell viability. The DEPArray system (Silicon Biosystems, Bologna, Italy) is a highly advanced system that allows single-cell isolation based on dielectrophoresis utilizing microfluidic cartridges. The DEPArray base is a microelectronic, active silicon substrate with embedded control circuitry for addressing over 80,000 individual DEP cages. These individual DEP cages enable manipulation and isolation of single cells. This has been

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Table 2. Combined Enrichment and Separation of CTCs
used to effectively separate labeled cell lines from peripheral blood [56,57] as well as to guide multiple binding of singularly-moved microspheres to a single tumor cell [58]. The ability to isolate and collect a single desired cell holds multiple advantages over other cell separation methods. The DEPArray offers this capability along with gentle cell manipulation, which results in single viable cells. In addition to single cell collection, cells of choice can be brought into contact with other cells to examine intercellular interactions.

Assays Combining both Enrichment and Detection of CTCs. Current assays of interest for CTC detection that combine an enrichment step and a detection method include the CellSearch System (Veridex, LLC, Warren, NJ, USA), the ISET (RareCells, Paris, France), the AdnaTest (AdnaGen AG, Langenhagen, Germany), EPISPOT (University of Mountpellier, Montpellier, France) and the Ariol system (Leica Microsystems, Buffalo Grove, IL, USA). These techniques are summarized in Table 2.

CellSearch. The US Food and Drug Administration (FDA) has approved the CellSearch system for clinical use to detect CTCs in peripheral blood. The CellSearch system was approved by the FDA in January 2004 for use in a clinical setting to predict outcomes for metastatic breast cancer patients [59–61]. In November 2007 and February 2008, the CellSearch system was also granted FDA approval to aid in monitoring colorectal [10] and prostate cancer patients [11]. Enrichment: The CellSearch immunomagnetically enriches cells expressing the epithelial cell adhesion molecule (EPCAM) using a ferrofluid containing anti-EPCAM antibodies (Kagan, M. 2002). Detection: Cells are then stained with a combination of anti-cytokeratin and anti-CD45 fluorescently-conjugated dyes [62]. Enumeration of cells expressing cytokeratin is accomplished by immunofluorescence (IF) [63]. Although CellSearch reliably captures CTCs, only cells expressing EpCAM and CKs are detected. Limitation: CTCs expressing non-epithelial phenotypes or that have undergone EMT and no longer express EpCAM or CKs are not detected by CellSearch.

ISET. A commercially available ISET (isolation by size of epithelial tumor cells) system (RareCells, Paris, France) has been described, and a study was conducted to compare the isolation capability of ISET to that of the CellSearch system [64]. Enrichment: The first step in ISET is enrichment for tumor cells based on their larger size. Cells are enriched by blood filtration through filtering membranes with calibrated pores 8 μm in diameter [32,65]. Detection: Enriched cells are then stained on the filter for cyt morphological examination or immunocytochemistry. ISET was reported to be capable of isolation of a single tumor cell in 1 mL of peripheral blood [32]. Concordant results between CellSearch and ISET were obtained in 55, 60, and 20% of the patients with breast, prostate, and lung cancers, respectively [64]. Limitation: ISET is limited because the size of CTCs varies greatly, and detection is based on epithelial markers.

AdnaTest. Another commercially-based assay developed for the detection of CTCs in patients with prostate, breast, and colon cancers is the AdnaTest by AdnaGen AG (Langenhagen, Germany). Enrichment: The AdnaTest utilizes enrichment of EpCAM-expressing cells that are isolated by magnetic separation. Detection: CTCs are detected by an RT-PCR assay to identify putative tumor-associated transcripts [66]. A recent comparison of the AdnaTest to the CellSearch system demonstrated concordance between the two, and it also reported that the AdnaTest improved the overall CTC detection rate and permitted the assessment of genomic markers in CTCs [66]. Limitation: False positives are possible due to contaminating nucleic acid, and cells are no longer viable following identification.

Microfluidic Devices. The CTC-Chip is based on a unique microfluidic platform capable of efficient and selective separation of viable CTCs from peripheral whole blood samples. The CTC-chip is mediated by the interaction of target CTCs with antibody (EpCAM)-coated microposts under precisely controlled laminar flow conditions, and without requisite pre-labeling or processing of samples [67]. To increase the number of cell-surface interactions with the antibody-coated device, a second-generation device referred to as the Herringbone chip (HB-chip) [68] was developed. The HB-chip was designed to allow thorough mixing caused by herringbone-induced microvortices, which disrupt the laminar flow streamlines of the cells. The HB chip successfully isolated CTCs from 14 of 15 (93%) patients with metastatic prostate cancer and allowed RNA-based detection of a rare tumor specific fusion transcript, TMPRSS2-ERG [68]. Cells are reported to be viable following isolation, but this microfluidic platform is currently limited to detection of CTCs that express EpCAM.

EPISPOT. Another technique for the detection of specific secreted proteins, epithelial immunospot (EPISPOT), is an adaptation of the enzyme-linked immunospot assay. EPISPOT detects only viable tumor cells and proteins secreted at the level of individual cells. An immunospot will only be observed by a viable cell because cell culture is required for enough secreted marker protein to be observed [69]. Enrichment: CTCs are first enriched from whole blood using a magnetic CD45 depletion followed by a magnetic CXCR4 positive selection. Detection: CTCs are then positively identified by an ELISPOT assay to detect MUC1 circulating...
tumor antigens mucin 1 (MUC1) or prostate-specific antigen (PSA). CTCs were detected by MUC1 or PSA and identified in the majority of patients with metastatic breast (100%) and prostate (83.3%) cancer but not in healthy controls [69-71]. Limitation: Only cells expressing CXCR4 are captured, limiting the heterogeneity of CTCs captured. Additionally, some CTCs may not survive the transition from the blood stream to in vitro culture, thus preventing their detection.

**Ariol system.** The Ariol system (Leica Microsystems) combines image capture using a Leica DM6000 microscope with quantitative clinical breast panel analysis. Enrichment: Whole blood is first lysed to remove RBCs, then enriched for CTCs using anti-CKs alone or with anti-EpCAM immunomagnetic antibodies [72]. Detection: The enriched cells are then subjected to immunocytochemistry, identifying cells positive for CK 8, 18, and 19, as well as cells negative for CK 45. The advantage of this method is that both EpCAM-positive and negative (expressing CKs) cells are detected. Limitation: The Ariol system is limited because the cells are no longer viable after separation.

**Discussion**

Because most CTC detection relies on epithelial markers [19,20], populations of CTCs with a non-epithelial phenotype are not detected. One possible reason for CTCs not expressing an epithelial phenotype is that they have undergone epithelial-to-mesenchymal transition (EMT). Metastatic potential is thought to increase once a cell has experienced EMT. The EMT process causes cells to have increased motility, invasiveness, and production of ECM components. This process is associated with up-regulation of vimentin, β4 integrins, α5β1 integrin, and αVβ6 integrin. Cells also experience a down-regulation of E-cadherin, EpCAM, and cytokeratins (CKs) [73-75]. Therefore, detection techniques that are based on EpCAM or CK expression likely do not detect CTCs that have experienced EMT. Characterization of CTCs remains, in large part, limited to those with epithelial phenotypes. More comprehensive detection techniques are required to ensure that all CTCs (independent of epithelial phenotype) are detected.

In addition to limited detection of CTC subtypes, another limitation to CTC capture is a lack of detection techniques resulting in reduced cell viability. The ability to isolate and manipulate viable CTCs of interest would allow them to be characterized beyond simple enumeration. This would provide additional markers to be identified, enabling the capture of a more comprehensive collection of CTCs. Additionally, because CTCs likely have heterogeneous metastatic phenotypes, the ability to isolate a single CTC would allow differences in CTCs to be studied and the most dangerous phenotypes to be characterized. Possessing knowledge about CTCs beyond total enumeration would dramatically increase their clinical utility.

Recent studies have revealed that there is heterogeneity within CTCs between patients and even within CTCs from a single blood draw [76]. Being able to subtype CTCs and identify metastatic potential for each subtype would provide clinicians with valuable information. Subtypes could also potentially be characterized to predict the site of metastatic invasion and the most effective treatment regimen.

Many new techniques to isolate and detect CTCs have been developed in the past decade. Although most of these methods are highly specific and sensitive, no extensive studies have been carried out to compare their efficacies. A very limited number of studies have compared results between a new cell separation technology and CellSearch [64,66,77,78]. New criteria should be established to address the lack of standardization in the field.

Desired qualities in a CTC separation technique include the ability to isolate rare cells of interest in great purity from vast quantities of unwanted hematopoietic cells, resulting in viable CTCs. Because most methods of separation result in non-viable cells, there is a great need to develop gentle methods of cell separation to be used for research purposes. A gentle separation method would provide the opportunity to culture CTCs *in vitro*, which could lead to an enormous amount of clinically useful information.

Currently, the clinical utility of CTCs is based on the enumeration of CTCs possessing an epithelial phenotype. Clinical utility would increase tremendously if comprehensive sets of CTCs could be captured and CTC subgroups with known metastatic
potentials established. The metastatic potential for each subgroup could be determined following analysis of patient samples; instead of simple enumeration, physicians could provide patients with a relative distribution of CTCs and their specific metastatic potential. This would provide physicians with information that could be used to accurately predict outcome, as well as the site of future metastatic lesions. This information would also help guide clinicians in selecting the most effective treatments for individualized patient therapy.

References


