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# Detection and isolation of circulating tumor cells: Principles and methods

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ABSTRACT

Efforts to improve the clinical management of several cancers include finding better methods for the quantitative and qualitative analysis of circulating tumor cells (CTCs). However, detection and isolation of CTCs from the blood circulation is not a trivial task given their scarcity and the lack of reliable markers to identify these cells. With a variety of emerging technologies, a thorough review of the exploited principles and techniques as well as the trends observed in the development of these technologies can assist researchers to recognize the potential improvements and alternative approaches. To help better understand the related biological concepts, a simplified framework explaining cancer formation and its spread to other organs as well as how CTCs contribute to this process has been presented first. Then, based on their basic working-principles, the existing methods for detection and isolation of CTCs have been classified and reviewed as nucleic acid-based, physical properties-based and antibody-based methods. The review of literature suggests that antibody-based methods, particularly in conjunction with a microfluidic lab-on-a-chip setting, offer the highest overall performance for detection and isolation of CTCs. Further biological and engineering-related research is required to improve the existing methods. These include finding more specific markers for CTCs as well as enhancing the throughput, sensitivity, and analytic functionality of current devices.



**Contents**



## 1. Introduction

Despite all efforts, cancer still remains a principal cause of death worldwide. In 2008, cancer claimed 7.6 million lives (~13% of all human deaths), and this number is expected to pass 13 million in 2030 (Ferlay et al., 2008; Jemal et al., 2011). According to the World

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Health Organization, however, at least 30% of these deaths are preventable. Early diagnosis and treatment of the primary tumor, which may prevent its subsequent metastasis, and development of more efficient therapies against the metastasized cancer, which accounts for 90% of cancer-related mortalities, are believed to be the key factors to win the *War on Cancer*. In particular, the efficiency of existing anti-metastatic therapies is mainly hampered by the heterogeneity of cancer cells as well as their peculiar interactions with the secondary host organ. Personalized targeted therapies that can be prescribed dynamically and according to the existing genotype and phenotype of cancer cells may significantly improve the quality of treatment (Kaiser, 2010). However, even if the metastatic lesions are detected and anatomically accessible for sampling, performing multiple regular biopsies is an impractical task. CTCs,[1] which are cancer cells detached from a primary or secondary tumor and entered the circulation, could be ideal specimens that: a) can be obtained easily and regularly, and b) provide the real-time single-cell-level data required for the effective identification of therapeutic targets.

In addition to their application in pharmaceutics and drug development, CTCs can be readily exploited in basic cancer research where the majority of the existing knowledge is based on mice models and cancer cell lines, both of which may not correctly represent the cancer problem (Ledford, 2011). For instance, single cell profiling of CTCs from breast cancer patients and its comparison with several breast cancer cell lines has shown considerable molecular differences between these cells, questioning the usefulness of studies performed on immortalized cell lines (Powell et al., 2012). Furthermore, histological analysis of the primary tumor samples may not be as useful, given that metastatic cancer cells could be genetically different from primary tumor cells. For example, CTCs detached from HER2-negative primary tumors in some breast cancer patients become HER2-positive, indicating that cancer cells experience further mutations after breaking off from the primary tumor (Fehm et al., 2010). Hence, studying CTCs can shed more light on the transient and still not clearly understood phase of cancer by which the malignancy metastasizes to other organs. CTCs may also improve our understanding about the natural selection process of MDR[2] and how it is related to major events in the metastatic cascade or stem cell properties in CTCs (Barrière et al., 2012; Kong et al., 2011; Scheel and Weinberg, 2012; Singh and Settleman, 2010; Szakács et al., 2006). In particular, the identification and characterization of CTCs with characteristics of CSCs[3] which may represent the true progenitors of metastatic tumors has become a topic of attention (Gupta et al., 2009; Visvader and Lindeman, 2012). The CSC model for carcinogenesis and metastasis is built on a hierarchical framework which predicts that tumor-derived cells with tissue progenitor characteristics, colloquially termed CSCs, can initiate and drive tumor spread through their intrinsic self-renewal capacity and the ability to maintain the tumor by giving rise to different types of non-CSCs (Reya et al., 2001; Visvader and Lindeman, 2012; Wicha et al., 2006). It has been suggested that it is during the differentiation of CSCs that epigenetic factors could lead to the emergence of chemorefractory cells that are responsible for the MDR effect in many cancers (Singh and Settleman, 2010). Although it has been assumed that CSCs are extremely rare (0.0001–0.1% of tumor cells) (Eaves, 2008), some studies have identified CSC properties in up to 25% of tumor cells (Gupta et al., 2009). A possible explanation for such a discrepancy is the difference in xenograft models used to assess the tumor forming capability of these cells. Identification and molecular analysis of circulating CSCs, which could be different from those residing in primary tumors, may provide additional insight into the nature of these cells. If accepted, the CSC model may realign many of the previously held notions on the classic clonal evolution theory of cancer development. A direct consequence of such a model would state that by targeting CSCs, metastasis can be managed (Clarke et al., 2006).

Aside from their applications in basic and translational cancer research, the frequency of CTCs in the PB[4] has been proposed as an accurate and less invasive clinical biomarker for diagnostic, prognostic, and pharmacological purposes (Bidard et al., 2010; Cristofanilli et al., 2004; Doyen et al., 2012; Krebs et al., 2010; Marrinucci et al., 2012; Mavroudis, 2010; Miller et al., 2010; Pantel and Alix-Panabières, 2007; Pantel et al., 2009; Paterlini-Brechot and Benali, 2007; Pierga et al., 2012). The haematogenous spread of cancer can be an early event in carcinogenesis, meaning that the growth of a primary tumor and a metastatic lesion could happen in parallel (Engel et al., 2003; Pantel and Brakenhoff, 2004). For instance, in nearly 5% of breast cancer patients, at least one overt metastasis is detectable at the time of initial tumor diagnosis, while 30–40% of patients may have developed occult metastases (Cristofanilli, 2006). Thus, CTCs may be detected in the PB even before the symptoms of the primary tumor are revealed and can be employed for early diagnosis of cancer (Alix-Panabières et al., 2007; Cristofanilli et al., 2004; Hüsemann et al., 2008; Kohn and Liotta, 1995; Pantel and Alix-Panabières, 2007).

Moreover, numerous prospective studies on patients with metastatic cancers of different organs have indicated that a higher number of CTCs at the baseline or any time during the therapy is associated with a shorter PFS[5] and OS[6] (Cristofanilli et al., 2004, 2005; De Giorgi et al., 2012; Doyen et al., 2012; Hiltermann et al., 2012; Hou et al., 2012; Iinuma et al., 2011; Krebs et al., 2010; Nieva et al., 2012; Sleijfer et al., 2007; Vona et al., 2004; Zhang et al., 2012). The prognostic value of CTCs has also been investigated in patients with primary non-metastatic cancers, where the presence of as low as one CTC per 7.5-mL sample prior to tumor resection has been correlated with a reduced OS (Lucci et al., 2012). Similar results have been reported when $\geq 1$ CTCs have been detected in a 30-mL sample (Franken et al., 2012). For such patients, CTC count may also be useful as a surrogate marker to assess the relapse risk after tumor removal (Allen-Mersh et al., 2007; Franken et al., 2012; Krell and Stebbing, 2012; Lucci et al., 2012; Yates et al., 2012).

CTC enumeration can also be exploited as a surrogate endpoint to evaluate the efficiency of anti-metastasis therapies (Devriese et al., 2011; Pantel and Alix-Panabières, 2007; Sleijfer et al., 2007). In other words, instead of using serological markers that occasionally lack the required sensitivity and specificity (e.g., due to a prolonged "spike" that is often observed in the marker level after administration of the drug (Hayes and Smerage, 2008)), or waiting for up to a few months to determine the response of a particular treatment using radiographic imaging, regular monitoring of CTC counts has been suggested as a rapid and accurate method for evaluating the treatment response (Devriese et al., 2011; Pachmann et al., 2008). A prospective randomized study run by Southwest Oncology Group (SWOG-S0500) is currently in progress, aiming to verify the suitability of changing therapies according to CTC counts.

Since PB sampling is performed easily, CTCs (i.e. liquid biopsy) can be frequently counted and analyzed, without the increased invasiveness, cost, and often low efficiency associated with other clinical assays (Pierga et al., 2012; Punnoose et al., 2010; Yu and Cristofanilli, 2011). For instance, in a study involving 138 breast cancer patients, inter-reader variability for the radiological assessment of the tumor and the associated CTC count has been reported to be 15.2% and 0.7%, respectively, which indicates the *precision* of CTCs as clinical biomarkers (Budd et al., 2006). Moreover, the median OS of patients with radiologically favorable prognosis but unfavorable CTC counts has been significantly shorter compared to that of patients with the same radiological prognosis and favorable CTC counts (15.3 versus 26.9 months). Also, patients

[1] Circulating tumor cells.
[2] Multidrug resistance.
[3] Cancer stem cells.
[4] Peripheral blood.
[5] Progression free survival.
[6] Overall survival.