

## Clinical Significance of Circulating Tumor Cells in Pancreatic Cancer

Guangfu Li<sup>1,2\*</sup>, Yue Huang<sup>1,2</sup>, Yariswamy Manjunath<sup>1,2</sup>, Eric T Kimchi<sup>1,2</sup>, Jussuf T Kaifi<sup>1,2</sup> and Kevin F Staveley-O'Carroll<sup>1,2,3\*</sup>

<sup>1</sup>Department of Surgery, University of Missouri, Columbia, Missouri, United States of America

<sup>2</sup>Ellis Fischel Cancer Center, University of Missouri, Columbia, Missouri, United States of America

<sup>3</sup>Department of Molecular Microbiology and Immunology, University of Missouri, Columbia, Missouri, United States of America

\*Corresponding authors: Guangfu Li, Ph.D, DVM, Assistant Professor, Department of Surgery, Ellis Fischel Cancer Center, The University of Missouri, One Hospital Drive, Columbia, Missouri 65212, United States of America, Tel: 573-882-8454; Fax: 573-884-6054; E-mail: liguan@health.missouri.edu

Kevin F Staveley-O'Carroll, M.D, Ph.D, Professor and Chair of Surgery, Director of Ellis Fischel Cancer Center, The University of Missouri, One Hospital Drive, Columbia, Missouri 65212, United States of America, Tel: 573-882-8454; Fax: 573-884-6054; E-mail: ocarrollk@missouri.edu

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### Abstract

Pancreatic cancer (PC) is a highly aggressive malignancy with limited therapeutic options. Early and accurate detection is critically important for successful cancer treatment. Currently, the most widely used and best validated marker for pancreatic cancer is carbohydrate antigen 19-9 (CA 19-9) and carcinoembryonic antigen (CEA). However, inadequate sensitivity and specificity limit the use of CA 19-9 and CEA in the early screening and diagnosis of pancreatic cancer. During past ten years, circulating tumor cells (CTCs) as a new liquid biomarker have received enormous attention in cancer diagnosis and management. Although CTCs are extremely rare and challenging to be isolated, their clinical significance has extensively been investigated in numerous clinical trials. In this review, we summarize the CTC application in translational pancreatic cancer research and its outlook in the future, specifically evaluating its clinical potential in pancreatic cancer diagnosis, prognosis, and treatments. Development of sensitive, specific, and reliable methods is expected towards a major impact on our ability in cancer management.

**Keywords:** Pancreatic cancer (PC); Circulating tumor cells (CTCs); Biomarker; Clinical trials

### Introduction

Pancreatic cancer (PC) is a highly aggressive malignancy with an average 5-year survival rate of <6% worldwide [1,2]. The most common type is pancreatic ductal adenocarcinoma (PDAC), representing around 90% of PCs [3]. According to the latest cancer statistics report from the American Cancer Society which is published in CA: A Cancer Journal for Clinicians [3], about 53,000 new cases of PC will be diagnosed in the US in 2016, causing around 42,000 deaths. PC is expected to surpass colorectal cancer to become the third leading cause of cancer-related death in the United States if current trend continues. Frequent and early dissemination of the disease, drug resistance, as well as late detection result in poor prognosis of PC patients [4]. The lack of progress in primary prevention, early diagnosis, metastases detection, and treatment underscores more efforts is needed in PC research [5,6].

Current biomarkers are insufficient in PC clinical management. Serum or tissue tumor markers have been proposed for use in clinical practice to predict prognosis, monitor response to treatment, and help detect recurrence or metastases. Among these biomarkers, carbohydrate antigen 19-9 (CA 19-9) [7] and carcinoembryonic antigen (CEA) [8] are the only blood-based biomarkers which are most widely investigated in clinical application. However, elevation of serum CA 19-9 and CEA is mostly found in patients with advanced and incurable PC. In addition, elevated levels of these tumor markers can be detected in individuals without PC. Low sensitivity and specificity limit their application for clinical screening. In clinical

practice, these tests are most useful to predict treatment response and cancer progression. Several other markers have been studied. Some studies have shown that CA19-9 and CA242 have similar sensitivity in the diagnosis of PC, which is higher than CEA [9]. However, no single marker has been shown to be better than CA19-9 [10].

Circulating tumor cells (CTCs) is emerging as one of the most promising liquid biomarkers with broad clinical application in cancer management. Thomas Ashworth, an Australian physician, firstly found CTCs in the blood of a patient with metastatic cancer in 1869. These cells are similar to the primary tumor cells identified in the blood vessels of autopsied cancer patients [11]. Sampling of peripheral blood CTCs provides a relatively non-invasive and real-time means to easily obtain reproducible liquid biopsy. These advantages highlight CTC's important value in clinical application [12]. Unceasing studies demonstrate that CTC is extremely rare as one milliliter of human blood carries approximately one billion red blood cells, seven million white blood cells, and 300 million platelets, but only about 1-10 CTCs [13]. In addition, no unique or CTC-specific markers can be used to isolate these cells. Therefore, detection and characterization of CTCs are still a challenging task. During the past more than ten years, technique advancement in the field of CTC has been taking place. Studies on CTCs have received enormous attention [14]. So far, large patient series of breast, prostate, lung, colon cancer, and PC have been testing for CTCs [15,16]. Searching for the term "circulating tumor cell" at <https://clinicaltrials.gov/> yields 823 clinical studies. Among them, 23 are of pancreatic cancer. Here, we categorize these ongoing or completed CTC clinic trials in PC, analyzing the reported results, and evaluate their potential in clinical utility towards advancing their future translational cancer research.

### Rapid expansion of CTC clinical trials in PCs

CTCs are the cells that shed into the vasculature from a primary tumor or metastases and circulate in the bloodstream. As a new real-time and liquid biomarker, CTCs provide an ideal means to increase our knowledge in tumor formation, progression, and metastasis. In addition, detection of CTCs can improve the early tumor detection and diagnosis, guide therapeutic choices. Therefore, the value of CTC as a new effective and reliable tool has received enormous attention and becomes one of the most active areas of translational cancer research.

In 2006, Dr. Sergeant and his colleagues registered the first CTC trial in PC in clinicaltrials.gov (NCT00495924). The aim of this study is to develop a CTC gene signature for assessing its prognostic relevance with surgical operation in PDAC patients [17]. Using FACS-based method, CTCs was enriched from the blood of 10 patients who underwent surgery for PDAC. Total RNA was isolated from four

different samples including CTCs, hematological cells (G), original tumors (T), and non-tumoral pancreatic control tissues (P). Whole genome microarray detected 8,152 genes, their functional analysis was conducted with 'Ingenuity Pathway Analysis' software and AmiGO. Finally, a CTC gene signature was developed and validated with the nCounter system on expression data of 78 primary PDACs. The results demonstrate that the defined cell motility gene signature in the primary tumor can predict survival of patients undergoing surgical resection for PC. Thereafter, more and more CTC clinical trials were requested and conducted. From 2001 to present, in total 23 registered CTC studies in PC were found in clinical trial (Table 1). Among them, around 80% trials were performed after 2010 (Figure 1). This rapid increase in CTC clinical trials for PC is similar to translational research in other types of cancers. The rapidly rising trend highlights the value, importance, and clinical significance of CTCs.

Registered No.	Clinical Utility	Start year	Sponsor	Status
NCT00495924	Early tumor recurrence	2006	Katholieke Universiteit Leuven	Completed
NCT00898781	Tumor progression	2007	Mayo Clinic	Terminated
NCT00524069	Neoadjuvant therapy efficacy	2007	Roswell Park Cancer Institute	Withdrawn
NCT00967603	Chemotherapy efficacy	2008	IRCCS San Raffaele	Completed
NCT01919151	Tumor initiation and progression	2008	Oslo University Hospital	Unknown
NCT01116791	Chemotherapy efficacy	2010	Baki Topal, University Hospital, Gasthuisberg	Terminated
NCT01313442	Genetic profiling	2011	National Cancer Institute (NCI)	Recruiting
NCT01474564	Genetic profiling	2011	Memorial Sloan Kettering Cancer Center	Recruiting
NCT01528774	Tumor progression	2012	Wolfram Samlowski, Comprehensive Cancer Centers of Nevada	Active, not recruiting
NCT01783054	Genetic profiling	2012	Medical University of South Carolina	Terminated
NCT02406846	Surgical and chemotherapeutic efficacies	2013	Fuda Cancer Hospital, Guangzhou	Completed
NCT01915225	Tumor diagnosis and treatment	2013	National Cancer Institute (NCI)	Recruiting
NCT02305810	Tumor progression	2013	European Institute of Oncology	Recruiting
NCT02150746	Surgical efficacy	2014	University of Florida	Completed
NCT02072616	Early diagnosis	2014	University Hospital, Rouen	Recruiting
NCT02080650	Comparison of CTC detection technology with c-MET and EpCAM	2014	Duke University	Recruiting
NCT02707159	Chemotherapy efficacy	2014	Helse Stavanger HF	Recruiting
NCT02349867	Neoadjuvant therapy efficacy	2015	Virginia Commonwealth University	Recruiting
NCT02555735	Chemotherapy efficacy	2015	Adera Labs, LLC	Recruiting
NCT02451384	Surgical efficacy	2015	Shanghai Zhongshan Hospital	Recruiting
NCT02838836	Early detection and genetic profiling	2016	University of Missouri-Columbia	Recruiting
NCT02335151	Efficacy of anesthesia	2016	University of Zurich	Not yet recruiting

**Table 1:** CTC clinical trials in pancreatic cancer.

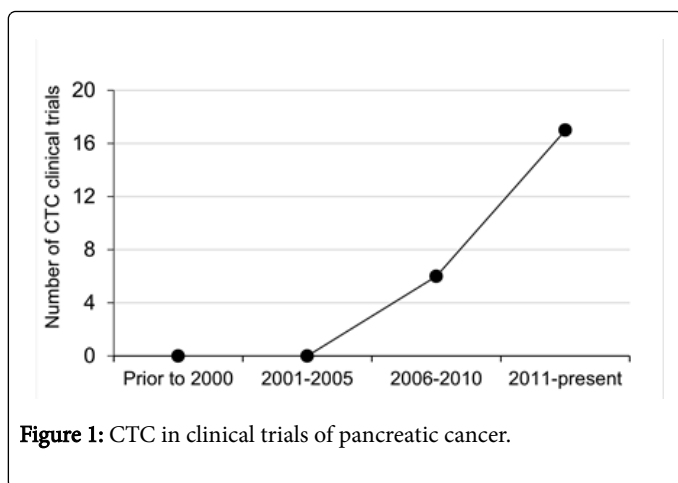


Figure 1: CTC in clinical trials of pancreatic cancer.

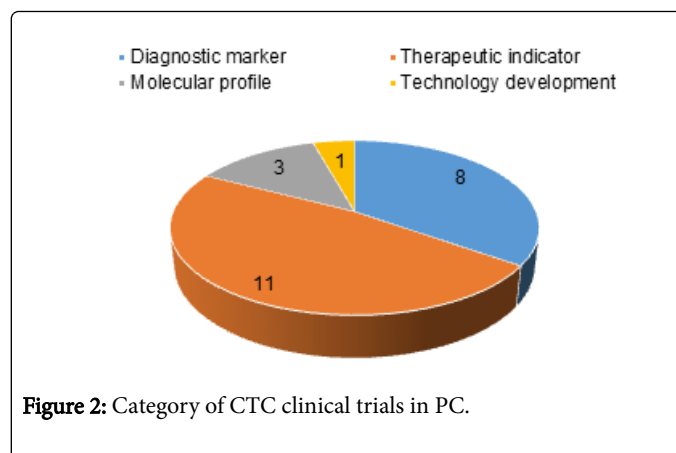


Figure 2: Category of CTC clinical trials in PC.

### Biological and molecular characteristics of CTCs in PC

Our knowledge of CTCs is still very limited. Generally, CTCs are more likely to be detected in advanced tumors due to higher tumor burden. However, it was reported that CTCs could be detected in very early stage of cancer without detectable tumors [18]. Considering very short half-life [19], CTCs is assumed to capably reflect the current status of primary tumors or secondary metastatic tumors. One study group conducted the first single-cell RNA sequencing for CTC from a mouse model of PC, and identified aberrations in the WNT pathway which might potentially be involved in the metastatic cascade [20]. This finding was verified by their following studies in patients with PC [21]. Another group recently reported that 200-fold increase in the mRNA expression of plakoglobin was detected in clustered CTCs in patient with PCs compared with single CTCs [22]. These completed and ongoing biological and molecular characterization of CTCs provide new insights into pancreatic cancer and its metastasis.

### Categories of CTC clinical trials in terms of clinical objectives

Currently, the clinical management of PC relies on the relatively few predictive markers including bloodborne CA 19-9 and CEA. However, these markers don't fulfill the requirement for early detection of tumor incidence and metastasis as well as a predictor of patient's response to treatments. Discovering of CTCs offer the promising tool to overcome these barriers by assessing tumor biology in real time. In terms of the objectives, a total of 23 registered CTC clinical trials fall into four major categories (Figure 2): 1) numeration of CTC as a diagnostic marker, 2) numeration of CTC as a therapeutic indicator, 3) molecular profiling of CTC, 4) new technique for CTC isolation. Among entire 23 trials, about 50% trials (11 studies) are used to predict the response to different therapies including surgical operation, chemotherapy, and adjuvant treatment. The second leading clinical application is detection of CTC for cancer diagnosis which account for 35% of total trials (8 trials). The third one is isolation of CTC for determining molecular profiles. Only one trial is to test a new CTC isolation technology with mesenchymal-marker ferrofluid (c-MET) by comparing with the FDA-approved EpCAM-based isolation. These trials demonstrate broad utility of CTC in translational cancer research.

### Diagnostic values of pancreatic CTC

Early and accurate detection of cancer is critical for successful cancer therapies. However, PC is difficult to find early. Pancreas is deep inside the body, early tumors cannot be seen or felt during routine physical exams. Patients usually have no symptoms until the tumors have already spread to other organs. Detection of CTCs within a routine blood specimen provides an opportunity to monitor cancer noninvasively, in essence a liquid biopsy [23]. Many reports have shown that pancreatic CTCs present in the peripheral blood of PDAC patients [24]. However, the reported results regarding the percentage of CTC positive patients and the number of CTCs per unit of blood vary dramatically between studies, which could be caused by diversity of the enrolled patients and the method difference used in CTC enrichment and detection [14]. Compared to other types of cancer, pancreatic cancer produces fewer CTCs in peripheral blood, evidenced by the lowest number of CTCs detected per unit of blood in PC among 12 different cancer types [24]. Catenacci et al. have reported that significantly more CTCs were detected in the portal vein blood than peripheral blood ( $118.4 \pm 36.8$  CTCs/7.5 mL vs.  $0.8 \pm 0.4$  CTCs/7.5 mL,  $p < 0.1$ ) [25]. Correspondingly, 100% patients were detected to be CTC positive (18 pancreatic cancer patients) with portal vein blood but only 4 out of 18 CTC positive patients were seen in peripheral blood. Similarly, 95% vs. 68% CTC detection in portal vein blood and peripheral blood was reported by another study group [26]. Also, detection of endoscopic ultrasound-guided fine needle aspiration biopsy led to diagnosis of PC in 77.8% patients while CTC detection in peripheral blood were only found in 55.5% patients [27]. These results suggest that portal vein circulation may serve as a barrier that blocks CTCs from entering systemic circulation. Thus, portal vein blood may be superior to peripheral blood in CTC detection. However, the safety of portal vein blood sampling needs to be further evaluated considering the high risk of thromboembolism in patients with PC [26]. Together, CTCs may have a significant potential in PC early screening and diagnosis.

### Therapeutic values of pancreatic CTCs

An increasing body of study has shown the difference of therapeutic response among different patients receiving same anticancer treatment. But, development of reliable tools to discriminate patients likely to benefit from specific therapies is still a challenging task. Yu et al. have created a pharmacogenomic model to predict the response of a pancreatic cancer patient to chemotherapeutic agents based on the

genetic expression signatures in pancreatic CTCs [28]. In a phase I study (NCT00429234), gemcitabine combined with dasatinib led to the reduced number of CTC in patients responded to the regimen compared to those without treatment response ( $0.5 \pm 0.0$  vs.  $3.85 \pm 1.57$  per 7.5 mL blood,  $p=0.052$ ) [29]. The results implicate the correlation of CTC number and treatment efficacy. However, large-scale further experiments with a well-designed control arm will be needed to validate this interesting result.

### Prognostic values of pancreatic CTCs

The potential of pancreatic CTCs to be used as a prognostic biomarker have been extensively studied. A number of studies have reported that a higher number of CTC was correlated with a poorer clinical outcome [30,31]. In 2013, 268 CTC-positive and 355 CTC-negative patients were enrolled by 9 study groups. Meta-analysis indicated that CTC-positive patients have a worse progression-free survival (PFS) and overall survival (OS) [32]. Soeth et al. reported that detection of CTCs in either bone marrow or peripheral blood was associated with a poorer survival in patients with well- or moderately-differentiated pancreatic cancer [33]. In the French LAP07 clinical trial, CTCs positivity has been linked to less differentiated tumors and a poorer OS [34]. A recently published study assessed the prognostic value of epithelial-like and mesenchymal-like CTCs [35]. The results showed the epithelial-like CTCs but not the mesenchymal-like CTCs were associated with a poorer survival while the mesenchymal-like CTCs was an indicator for early tumor recurrence. However, irrelevance of CTC with survival was reported in some studies or in different setting. A phase II clinical trial in metastatic PC (NCT00474812) indicated that the number of CTCs at baseline were not associated with OS [36]. In a clinical trial (NCT00495924), surgical operation was found to increase CTC number which was not associated with a poorer clinical outcome [37]. Similarly, Gall et al. has employed a no-touch isolation technique for pancreaticoduodenectomy. Although this new technique significantly reduced CTCs postoperatively compared to the standard technique, no survival benefits were observed [38]. Bissolati et al. has found that the incidence of liver metastasis was significantly lower in patients without detectable CTCs compared to patients with CTCs (8% vs. 53%,  $p=0.038$ ) [39]. However, the reduction of liver metastasis did not translate to survival benefits. In a clinical trial (NCT00495924), Sergeant et al. attempted to use gene expression signature of CTCs to predict survival for pancreatic cancer patients who underwent surgical tumor resection. The high co-expression of TGF- $\beta$ 1 and a group of 9 genes function in cell motility were found to be independent markers to predict the PFS and OS [17]. Taken together, many studies have reported promising results to support the use of CTCs as a prognostic marker in PC. However, more studies are needed to define the clinical values of different subsets of CTCs in the future.

### Challenges and future directions

CTC as a liquid biomarker is easily accessible and reproducible. Great effort advances its clinical application over the past decade. In addition to its inherent significance in cancer diagnosis and prognosis, molecular profiling of CTCs in a real time has provided new insights into the biology of tumor metastasis which may facilitate the development of "tailor-made" cancer treatment for an individual cancer patient. However, challenges still remain in CTC isolation and identification due to its extremely rare in the blood and technological limitation in isolation. Large-scale clinical data are still lacking. In

addition, different principles for identification of CTC generate diverse results which is confusing to the cancer research. Therefore, more sensitive, specific, and reliable methods for CTC identification and isolation as well as its downstream analysis are urgently needed. In addition, it has been reported that some critical processes including epithelial mesenchymal transition (EMT) are required for primary tumor cells to differentiate into CTCs [12]. In terms of the differentiation status, CTCs can be classified into different subpopulation including epithelial CTCs, mesenchymal CTCs, and hybrid (epithelial/mesenchymal) CTCs etc. [40,41]. Each subpopulation may have different clinical implication. It is postulated that only a specific subpopulation mediates tumor metastasis. Thus, characterization of CTC subpopulation will expand our understanding of CTCs and improve our ability in cancer management [42].

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### Conflict of Interest

The authors have declared that no conflict of interest exists.

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