Journal of Oncology Research and Treatments

James et al., J Oncol Res Treat 2020, 5:2

Research Article Open Access

Detection of Circulating Tumor Cells (Liquid Biopsies) Using Merisistm CTC Kit

Rani James, Santhosh Gopal, Vanitha KP and Kaushik DEB*

Merisis Therapeutics, DiponEd BioIntelligence, Karnataka, India

Abstract

Objective: Circulating tumor cells (CTC) has prognostic value in patients with solid tumors, such as advanced breast, colon, and prostate cancer. The main diagnostic and treatment methods for patients with cancer are gradually shifting from conventional standards to personalized techniques. At Diponed BioIntelligence, we developed a CTC isolation kit integrated with a miniaturized filter for CTC isolation.

Methods: Paired peripheral blood samples were collected from 20 cancer patients (10 suspects and 10 confirmed cases of cancer) to enumerate CTCs using Merisis™ CTC Kit. The results are compared with healthy controls.

Results: The number CTCs detected were ≥ 10/10ml of blood in Confirmed cancer cases using Merisis™ CTC Kit. The numbers of CTC's were ≤ 5 in healthy and suspected cancer patients.

Conclusions: The Merisis™ CTC Kit has a potential to isolate significantly more CTCs and CTC clusters in advanced stage cancer patients.

Keywords: Circulating Tumor Cell; CTC; Kit; Tumor; Liquid biopsy

Introduction

Isolation and detection of circulating tumor cells (CTCs) from human blood plays an important role in non- invasive screening of cancer evolution and in predictive therapeutic treatment. Innovative modern technologies are capable of performing previously unachievable tasks. Due to these developments, the main diagnostic and treatment methods for patients with cancer are gradually shifting from conventional standards to personalized techniques. Moreover, current diagnostic technology has led to the rapid development of precision medicine and molecular diagnostics. Here, we present the novel simultaneous separation and label-free analysis of circulating tumour cells CTCs in the blood specimens with high specificity and sensitivity [1-6].

Circulating tumor cells (CTCs) are living cancer cells separated from the primary tumor, which are responsible for the development and expansion of the metastasis form of cancer. The time depended evolution and molecular characterization of CTCs in the peripheral blood are crucial and non-invasive sources for the tumor diagnostics, cancer therapy selection, monitoring and prognosis [7].

As a liquid biopsy, these markers complement solid biopsies and have the advantage of being physically more accessible and patient-friendly than traditional tissue biopsies. This provides a possibility for prognosis prediction, closer monitoring of treatment response and disease progression, identification of drug targets, as well as an opportunity for early detection of recurrence [8].

Liquid biopsy techniques can provide real-time information regarding patient staging (metastatic vs. nonmetastatic) and the molecular profile of the tumor. Moreover, liquid biopsies can be repeated with the desired frequency for close monitoring of progress and treatment.

Materials and Methods

Study design and ethics statement

This prospective study was conducted to evaluate CTC enumeration using the Merisis™ CTC Kit in patients with cancer in a blinded experiment. CTCs and ctDNA were analyzed in blood samples collected from cancer patients and healthy controls. The study included 10 patients of suspected cancer, 10 patients of confirmed cancer and 15 healthy volunteers. The presence of CTCs was assessed individually according to their criteria before knowing any results from each other. The study inclusion criteria were patients with histopathologically confirmed cases of cancer. The institutional review boards of Gurushree Hospital approved the study protocol and all patients provided written informed consent.

Merisis™ CTC kit

Merisis Circulating Tumor Cells (CTC) isolation uses separation gel which efficiently separates CTC from peripheral blood based on density of CTC and the gel. The density of the separation gel is tailor made to suit for the efficient separation of circulating tumor cells from the whole blood. 10 ml whole blood was collected in CTC tube. The blood samples were transported at 0-4°C and subsequent analyses were performed within 24h after sample taking.

Merisis™ CTC Kit consists of a 13 ml glass tube with an inert gel barrier that act as unique separation medium. The two properties of CTC, i.e. low specify gravity and large size, greater than 10 µm-15µm (compared to other cells present in blood) makes it possible to achieve high recovery rates. 8 ml blood is collected by venipuncture. The tube containing blood is centrifuged at 3500 for 5 minutes. After centrifugation the RBC's are separated from the plasma. The CTC tube separates blood components based on specific gravity (Figure 1). The CTC tube contains gel that acts as a barrier, so that RBC with specific gravity >1.077 gm/ml will reach the bottom of the CTC tube after

***Corresponding author:** Kaushik DEB, Merisis Therapeutics, DiponEd BioIntelligence, Karnataka, India, Tel: +91 99005 20141; E-mail: deb@diponed.com

Received January 29, 2020; **Accepted** July 22, 2020; **Published** July 29, 2020

Citation: James R, Gopal S, Vanitha KP, Kaushik DEB (2020) Detection of Circulating Tumor Cells (Liquid Biopsies) Using Merisistm CTC Kit. J Oncol Res Treat 5: 149.

centrifugation. CTCs with specific gravity <1.047 gm/ml will reach to the top after centrifugation process. Extract the top layer containing CTC. The CTC's are the separated mainly based on differences in the size and deformability between CTCs and hematologic cells. As tumor cells (>8 μm) are larger than leukocytes, isolation by size of epithelial tumor cells (ISET) can be achieved using filtration to separate individual cells [9].

The collected supernatant is passed through the separation tube containing membrane filter which allows the passage of hematological cells. Figures 2 and 3 haematological cells are <8 μm, so it will pass through the filter. Now the supernatant contains only CTC. Merisis™ CTC tube uses membrane filter that are inexpensive and user-friendly method of enriching CTCs, thus enables the recovery and detection of CTCs on the basis of size-dependent CTC isolation.

The captured cells have also been tested for epithelial and mesenchymal markers and a substantial number of the cells (~86%) were positive, showing the advantage of size-based separation. CTCs were counted, identified as being cytokeratin positive and CD45 negative and EpCam positive. Genetic analysis, which can provide diagnostic and prognostic information, can also be carried out on

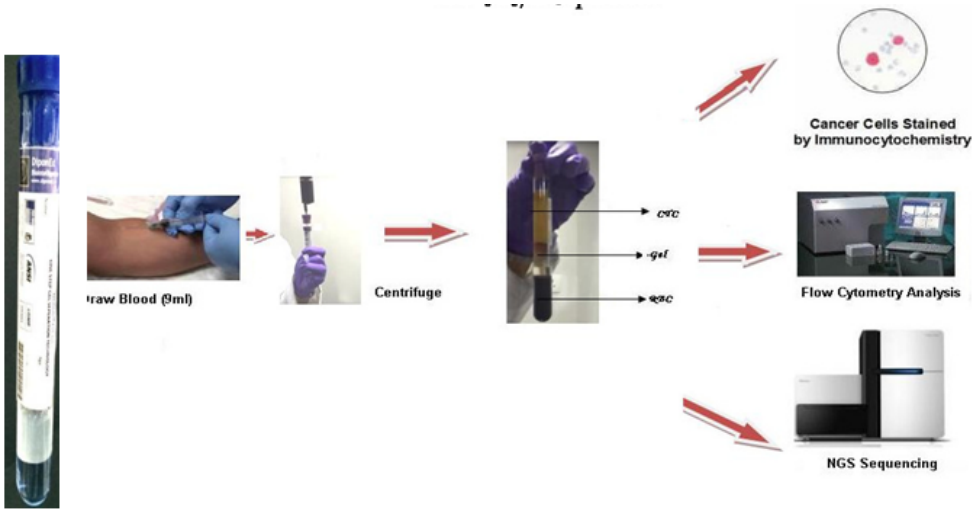

Figure 1: Merisis CTC/D Separation.

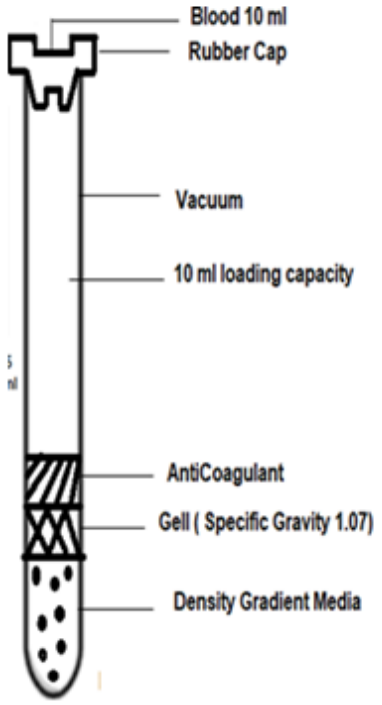

Figure 2: Specific gravity based separation.

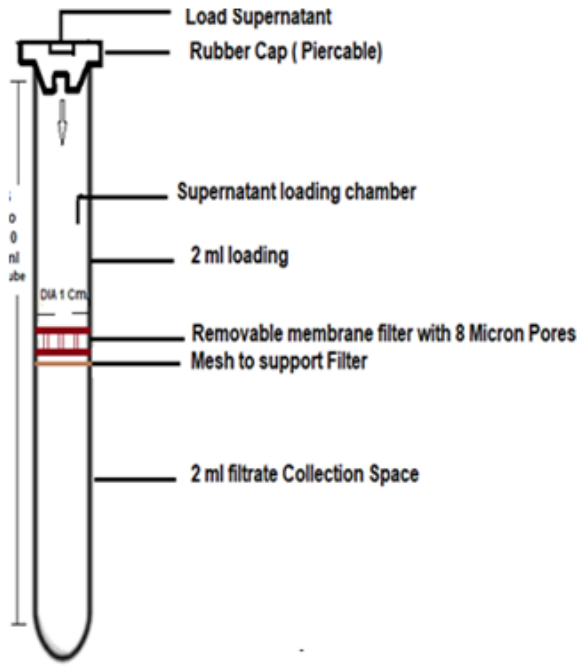

Figure 3: For size separation.

Type/Groups	No of patients	CTC count/10 ml blood
Suspects	10	4+/- 2 cells
Cancer patients	10	10+/- 2 cells
Healthy	15	5+/- 2 cells

Table 1: CTCs count in test subjects.

Key parameter	Merisis CTCs tube	Other enrichment techniques
Blood sample capacity	8 ml	10 ml
Sample preprocessing requirements	No need	Need
Cost of consumables and equipment	Less	High
Processing speed	Within 2 days	More than 1 week
Purity of CTCs	High	Low
Cell viability	>95%	80% - 90%
Capture efficiency	>99%	90% - 95%

Table 2: Advantages of Merisis CTC technology.

live cells. Ct DNA was isolated and are used to study gene expression profile by Real Time PCR (Figure 1).

Results

CTCs were detected in 10 +/-2 /10ml of blood using Merisis CTC kit. The number of CTC's were ≤ 5 in healthy and suspected patients. The results are shown in Table 1.

Discussion

Analysis of CTCs can save a patient from worsening the condition with unsuitable medications. Furthermore, the earlier they are detected, faster and better treatment options can be made available to the patient. It provides the basis of understanding mutations and genotypic changes of malignant cells and hence provides the best suitable targeted therapy. CTCs are multifunctional biomarkers and enable us to assess the patient serially along the treatment journey. They are potentially an alternative to invasive biopsies for detection, characterization and monitoring of non-hematological cancers [9-11].

Merisis™ CTC Kit with a density gradient polymer gel isolates cancer cells from normal cellsbased on specific gravity and size and by positive and negative selection markersusingRT PCR analysis.The purity of the CTC obtained is high with cell viability >95% compared to other enrichment techniques (Table 2).

The proposed approach challenges the current multi-steps CTCs detection methods in the terms of simplicity, sensitivity, invasiveness, destructivity, time and cost of analysis, and also prevents the defragmentation/damage of tumor cells and thus leads to improving the accuracy of analysis. The results of this research work show the potential of developed Merisis™ CTC Kit for the separation of tumor cells from whole blood samples in a simple and minimally invasive manner, their detection and molecular characterization using one single technology.

References

1. Danila DC, Fleisher M, Scher HI (2011) Circulating tumor cells as biomarkers in prostate cancer. Clin Cancer Res 17: 3903-3912.
2. Tanaka F, Yoneda K, Kondo N, Hashimoto M, Takuwa T,et al. (2009) Circulating tumor cell as a diagnostic marker in primary lung cancer. Clin Cancer Res 15: 6980-6986.
3. Alix-Panabieres C, Pantel K (2014) Challenges in circulating tumour cell research. Nat Rev Cancer 14: 623-631.
4. Nagrath S, Sequist LV, Maheswaran S, Bell DW, Irimia D, et al. (2007) Isolation of rare circulating tumour cells in cancer patients by microchip technology. Nature 450: 1235–1239.
5. Yu M, Stott S, Toner M, Maheswaran S, Haber DA (2011) Circulating tumor cells: Approaches to isolation and characterization. The Journal of cell biology 192: 373-382.
6. Pantel K, Alix-Panabieres C (2003) Real-time liquid biopsy in cancer patients: fact or fiction? Cancer research 73: 6384-6388.
7. Swaby RF, Cristofanilli M (2011) Circulating tumor cells in breast cancer: A tool whose time has come of age. BMC Medicine 9: 43.
8. Förnvik D, Aaltonen KE, Chen Y, George AM, Brueffer C, et al. (2019) Detection of circulating tumor cells and circulating tumor DNA before and after mammographic breast compression in a cohort of breast cancer patients scheduled for neoadjuvant treatment. Breast Cancer Res Treat 177: 447-455.
9. Hosokawa M, Kenmotsu H, Koh Y, Yoshino T, Yoshikawa T, et al. (2013) Size-based isolation of circulating tumor cells in lung cancer patients using a microcavity array system. PLOS ONE Volume 8: e67466.
10. de Wit S, van Dalum G, Terstappen LW (2014) Detection of circulating tumour cells. Scientifica (Cairo) :819362.
11. Krebs MG, Metcalf RL, Carter L, Brady G, Blackhall FH, et al. (2014) Molecular analysis of circulating tumour cells-biology and biomarkers. Nat Rev Clin Oncol 11:129-144.