Bioinformatics-Based Analysis of Circulating Tumor Cells: A Methodological Overview of Personalized Cancer Treatment

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***Abstract*—Circulating Tumor Cells (CTCs) are capable of providing valuable insights throughout the course of a patient's disease, even during the early stages of cancer. CTCs have recently gained significant interest for their potential for advancing personalized cancer treatment studies. Despite the challenges associated with their detection and analysis, emerging bioinformatics methodologies and technologies have enhanced the understanding of these cells and their role in cancer metastasis. This paper provides a methodological overview of these bioinformatics methodologies, particularly focusing on the computational analysis of CTCs and its implications for personalized cancer treatment strategies. Additionally, the paper highlights the importance of refining current techniques and need for a further development to overcome the limitations of liquid biopsies, such as their limited sensitivity in detecting low-abundance biomolecules. With this overview, we aim to show the transformative potential of CTC analysis combined with bioinformatics, providing valuable insights for future research and clinical applications in cancer immunotherapy and personalized medicine.**

***Keywords*—circulating tumor cells, analysis of CTCs, cancer immunotherapy, personalized cancer treatment, bioinformatics-based analysis**

I. Introduction

CTCs are capable of providing valuable insights throughout the course of a patient's disease, even during the early stages of cancer. This is achieved through a process often referred to as a liquid biopsy. This highly sensitive method allows for the extraction of these cells from a patient's blood sample, detecting CTCs even at extremely low concentrations [1]. The potential benefits of detecting and counting CTCs include more targeted and precise treatment, particularly for patients who had a primary tumor surgically removed [2].

Understanding and detecting CTCs, however, continue to present significant challenges, primarily due to their rarity among blood cells and their potential to directly interact with the immune system [3]. Tumor heterogeneity which refers to the genetic, phenotypic, or functional differences between cancer cells within a single tumor is also an issue, further complicates matters particularly in isolating and detecting CTCs [4].

Next-Generation Sequencing (NGS) technologies can sequence CTCs at an incredibly high speed, offering detailed insights into their genomic profile. Therefore, liquid biopsy when integrated with NGS technologies has critical role in detecting and analysing CTCs, contributing significantly to the early detection of cancer [5]. The use of liquid biopsy and NGS provide insights into the patient's cancer at a molecular level. This knowledge enables more personalized approaches to treatment, which can be adjusted based on real-time monitoring of tumor evolution and response to therapy.

However, the integration of these technologies generates a substantial amount of data that require advanced bioinformatics for accurate interpretation. The use of bioinformatics is crucial task, as it offers the ability to interpret this complex data and to know more about the molecular structure and characteristics of the CTCs. This knowledge aids in cancer diagnosis, tracking tumor evolution, and personalizing treatment plans. Furthermore, these approaches can evaluate the efficacy of chemotherapy and guide the selection of effective anti-cancer drugs.

Integrating liquid biopsy, next-generation sequencing, and bioinformatics provides a multifaceted approach to studying therapeutic targets, drug resistance, disease progression, or the genetic basis of the disease [6]. This process utilizes cutting-edge technologies and computational analysis to deliver crucial insights into diseases, particularly cancer.

In recent years, there has been a growing interest in bioinformatics methodologies and technologies utilized in the computational analysis of CTCs and their potential for designing personalized immunotherapy strategies. This paper reviews bioinformatics methodologies and technologies utilized in the computational analysis of CTCs, elucidating their implications for designing personalized immunotherapy strategies. We explore how

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these strategies inform personalized medicine efforts in cancer immunotherapy by analyzing and interpreting the complex biological data represented by CTCs. Through this review, we aim to show the transformative potential of integrating CTC analysis and bioinformatics and to provide valuable insights for future research and clinical applications in this rapidly evolving field.

II. Methodological Overview

A. Liquid Biopsy

The integration process begins with a liquid biopsy, a minimally invasive method for detecting circulating biomarkers such as circulating tumor DNA (ctDNA), CTCs, and exosomes, typically collected from biological fluids like blood, urine, or saliva. Liquid biopsy provides an opportunity for non-invasive cancer diagnosis and monitoring. After isolation of tumor compartments in body fluids (CTCs, ctDNA, exosomes), their material serves as the foundation for the subsequent processes, as the data gathered will be further analyzed and processed.

B. Sample Preparation and NGS

After isolation of any compartment of the liquid biopsy, the collected biomarkers are prepared for NGS for purification, concentration, and library preparation of DNA or RNA samples. Subsequently, these samples are sequenced using NGS technology, which supports high-throughput analysis for massively parallel sequencing, generating extensive data for each sample and yielding raw sequencing reads.

Single-cell RNA sequencing (scRNA-seq) [7] is a subarea of NGS which helps to analyze the transcriptomes of single-cells, facilitating the analysis of millions of cells in one single experiment [8]. When integrated scRNA-seq with a non-invasive method like liquid biopsy allows the examination of gene expression of CTCs at the single-cell level, which will provide an extensive view that helps provide additional insights into the heterogeneity of CTCs.

C. Bioinformatics Analysis

After sequencing, the raw sequencing data undergoes an extensive bioinformatics analysis. The analysis often starts with a quality control, where raw reads are scrutinized to remove or correct any low-quality reads or errors introduced during the sequencing process. Next alignment or mapping takes place for the cleaned reads to a reference genome. Subsequently, variant calling is conducted to identify the differences or mutations in the sequenced DNA compared to the reference genome. The final stage of bioinformatics analysis involves annotation and interpretation, which identifies mutations present in known genes, their potential effects on protein function, and their relevance to disease. The high level of noise and the complexity of scRNA-seq data make it difficult to interpret the results, requiring advanced bioinformatics tools and expertise.

III. Bioinformatics Methods for CTCs Analysis

The dissemination of Circulation Tumor Cells (CTCs) is a crucial step in carcinoma metastasis. These cells originating from primary tumors, shed, enter the bloodstream, and circulate throughout the body. Due to their small size and dependence on the tumor stroma for survival and support, they remain undetected for extended periods, and present a significant challenge in early cancer detection [9]. As these circulating tumor cells navigate through the body, they can spread to diverse areas and infiltrate both neighboring and distant host tissues [10]. There, they continue to proliferate, eventually leading to metastasis, further increasing the complexity of cancer treatment [11].

Despite the initial discovery of CTCs in 1869 [12], comprehensive and systematic studies on these cells, which are now considered to be critically important in cancer care [13] did not commence until much later, with significant advancements made in the early 2000s [14]. The techniques for CTC detection have significantly evolved since then, with current methods largely utilizing the next generation sequencing [15–17] and PCR-based approaches [18, 19]. These technological advancements have not only enhanced the understanding of cancer spread and progression but also facilitated the development of more effective cancer treatments.

Recent progress in bioinformatics has provided solutions for the challenges associated with the analysis of CTCs (Table I). As such, bioinformatic pipelines can have a transformative effect in the field of liquid biopsy. These developments also have the potential to improve the design of personalized immunotherapy strategies, offering new possibilities for cancer treatment.

In many studies, NGS is utilized for genetic profiling and characterization of CTCs. Gulbahce *et al.* [20] combined immunomagnetic enrichment and fluorescence-activated cell sorting for CTC isolation, followed by in-depth bioinformatic analysis of the genomic data. They enabled accurate detection and quantification of somatic mutations, contributing to the design of personalized combination therapies for metastatic cancer patients. Gkountela *et al.* [21] explored the role of DNA methylation changes in CTC clusters in promoting metastasis in breast cancer patients. They analysed bisulfite-sequencing data and suggested a potential therapeutic strategy using Na+/K+ ATPase inhibitors to disperse CTC clusters and inhibit metastasis. Ruan *et al.* [15] isolated CTCs from cerebrospinal fluid of patients with lung adenocarcinoma leptomeningeal metastases and then profiled using scRNA-seq. Through gene expression analysis, they characterized these cells and revealed key molecular pathways involved in cancer metastasis. Bertolini *et al.* [16] presented a bioinformatics method for analysing single-cell RNA sequencing data with its potential to process and interpret data from CTCs. The method provides a detailed molecular profiling of these cells, thereby helping to understand tumor heterogeneity, metastatic potential, and personalized response to therapy. Chiu *et al.* [22] analyzed data from single circulating tumor cells of prostate cancer patients

using single-cell RNA sequencing-based differential network analysis. Ting *et al.* [23], utilized unsupervised hierarchical clustering, and principal component analysis in a mouse model of pancreatic cancer. Through the use of single-cell RNA sequencing established unique expression profiles for CTCs, distinct from primary tumor cells and tumor-derived cell lines. They observed a significant heterogeneity among CTCs, as assessed by intracluster correlation coefficients. Xu *et al.* [24] utilized scRNA-seq to analyze the heterogeneity and evolutionary patterns of circulating tumor cells in solid tumors.

Some research has also benefited from machine learning, such as the work of Guo *et al.* [17]. They applied deep transfer learning to analyse and classify CTCs based on their original lesions, bridging the gap between primary cancer cells and CTCs. They also utilized scRNA-seq data for cell characterization, thus emphasizing the role of bioinformatics techniques in analysing and interpreting high-dimensional data. Miyamoto *et al.* [25] developed a unique multiclass SVM classifier trained on droplet digital PCR data derived from prostate cancer cell line and CTCs.

The application of bioinformatics methods for early cancer detection has also seen significant progress. Hou *et al.* [26] suggested a strategy that integrates minimally invasive liquid biopsies with the NGS and bioinformatics for the early detection, monitoring, and precise prognostication of pancreatic cancer. They focused on the potential of integrated biomarkers, which can be identified through extensive inspection of a comprehensive array of circulating molecules including CTCs, DNA, extracellular vesicles, and proteins. Similarly, Schissler *et al.* [27] developed an analytic framework using advanced bioinformatics methods to understand the dynamics of transcriptome within CTCs derived from prostate cancer patients. Their aggregation method improved the identification of differentially expressed pathways in individual cells potentially enhancing the precision of therapeutic decision-making processes in personalized cancer treatment. Keomanee-Dizon *et al.* [28] explored the use of liquid biopsies and bioinformatics in the high-definition single-cell analysis of CTCs. Their approach, which involved sophisticated image processing, offering significant promise for early cancer detection.

Focusing the prognostic potential of bioinformatics in liquid biopsy studies, Ramalingam and Jeffrey [29] presented an overview of the current challenges, future opportunities and the importance of bioinformatics and technological advancements in liquid biopsy studies. They particularly emphasized the potential of methodologies involving CTCs to provide real-time insights into tumor evolution and response to therapy. Kapeleris *et al.* [30] used bioinformatics methods like exome sequencing to profile and expand CTCs, facilitating the characterization of CTC cultures and confirming the presence of somatic mutations with mutational signatures consistent with non-small cell lung cancer. Guan *et al.* [31] used bioinformatics tools to analyze gene expression data to reveal gene expression differences between CTCs and primary tumors. Their analysis identified PSMC2 as a poor prognostic indicator in renal cancer, highlighting the value of bioinformatics and NGS technologies in cancer research, prognosis, and treatment planning. Valladares-Ayerbes *et al.* [18] discovered gastrointestinal cancer-specific markers for the detection of CTCs using bioinformatics and liquid biopsy technologies with the potential to identify patients at high risk of relapse or disease progression. Guan *et al.* [32] studied the correlation between CTCs, platelets, and immune-inflammatory cells in patients with renal cell carcinoma using liquid biopsy and bioinformatics technologies. Their analysis revealed that mesenchymal CTCs, the monocyte-to-neutrophil ratio, and staging could be used to predict the risk of postoperative metastasis in these patients. Yan *et al.* [33] explored the expression of immune checkpoint FGL1 in CTCs from hepatocellular carcinoma patients. Their findings reveal that FGL1 expression in CTCs is associated with poor survival and higher rates of metastasis.

In studies related to treatment response, Yin *et al.* [34] presented how NGS and liquid biopsy can enhance the assessment of neoadjuvant therapy response in patients with pancreatic ductal adenocarcinoma. By examining somatic mutations, circulating tumor cells, and circulating tumor DNA, the study points toward the possibility of early recurrence and reduced survival even in patients showing pathologic complete response. Gong *et al.* [35] applied NGS and bioinformatics to analyse gene activity in tumor and nontumor tissues of hepatocellular carcinoma patients. They suggest the significant influence of adjacent nontumor tissue on the colonization of CTCs after treatment.

IV. Discussion and Concluding Remarks

Metastasis, the primary cause of death in cancer patients, is a complex process. CTCs must overcome numerous challenges to successfully colonize distant organs, including penetrating distant tissues, evading immune defenses, adopting to new environments, and survive as potential tumor-initiating cells, eventually taking over the host tissue. Even though these difficulties, once metastases have formed, current treatments frequently fall short in providing sustained responses. Thus, there is a need for a more comprehensive understanding of the mechanisms of metastatic formation to develop effective prevention and treatment strategies for metastatic cancer.

The integration of bioinformatics and liquid biopsy technologies has provided promising prospects for cancer research, from early detection to treatment planning. Liquid biopsies could provide valuable insights for the origins and characteristics of CTCs, stepping towards personalized treatment strategies. However, the accuracy and reliability of liquid biopsies depend on extensive validation. Moreover, the performance of these biopsies can vary significantly based on the techniques used and the type and stage of the tumor. It’s also important to note that a single biomarker may not provide a comprehensive view due to potential limitations associated with specific diagnostic techniques.

TABLE I. COMPARATIVE OVERVIEW OF STUDIES UTILIZING BIOINFORMATICS APPROACHES IN THE ANALYSIS OF CTCS

Related Studies	Bioinformatics Approaches	CTC Analysis
15	scRNA-seq, clustering and expression analysis to define transcriptomic characteristics of the isolated CTCs from cerebrospinal fluid	Examination of CTCs on a molecular and transcriptomic level
16	Computational pipeline for the analysis of scRNA-seq including cell type identification, gene and pathway visualization, and in silico drug candidate identification	Detailed molecular profiling of CTCs
26	Integrated biomarker profiles for a more accurate and early diagnosis	Real-time profiling of CTCs
17	Single-cell RNA sequencing, deep transfer learning to analyze and classify CTCs based on their original lesions	Characterization of CTCs in liquid biopsy samples
27	Analysis of aggregated cell-cell statistical distances within biomolecular pathways	Utilization of CTCs to develop analytic methods that would identify differentially expressed pathways associated with therapeutic resistance
29	Variant calling, mRNA-seq, whole exome sequencing, high throughput droplet-based methods	Single-cell analysis of CTCs provided important information about tumor heterogeneity
18	Analyzing in silico data to select genes highly expressed in gastrointestinal cancers but not in hematopoietic-derived libraries	PKP3 and AGR2 were identified as potential markers for CTC detection in gastrointestinal cancer
28	Analysis of the high-throughput imaging data generated by the high-definition single-cell assay	High-definition single-cell assay designed to enrich for and image CTCs
30	Analysis of the exome sequencing data generated from the CTC cultures	CTCs obtained, specifically, from blood samples of patients
31	Gene chip analysis, differential gene expression analysis, GO/KEGG enrichment analysis, PPI analysis, to analyze the data and identify key genes and functions of CTCs	Analysis of the differences between the gene expression profiles of CTCs and primary tumors
32	Analysis of the differentially expressed genes between CTCs and normal tumor cells, identifying that the main function of these genes was related to platelets and immune inflammation	Evaluation of relationships and correlations among CTCs, inflammatory cells, and platelets in the blood of these patients
33	FGL1 expression evaluation and immune infiltration in tumors, and cross-referenced with liquid biopsy results	CTC detection as an alternative to tissue sampling for FGL1 expression, impacting HCC treatment and potential immunotherapy applications
34	NGS to detect somatic mutations	The presence of CTCs was found in the blood of patients for whom data was available, indicating the possible existence of minimal residual disease even in patients with a pathologic complete response (pCR). The presence of CTCs can be potentially used as an indicator of early recurrence and reduced survival
35	Analysis of gene expression data	Changes in nontumor tissue can affect the proliferation and colonization of CTCs post-treatment, such as after hepatectomy
22	Gene-gene correlation adjustments based on sample size, comparison of inter-state correlations, and construction of differential networks	Ability to handle sparse scRNA-Seq data from CTCs and illuminate differential gene regulation
23	Analysis of gene expression profiles of individual CTCs	Characterizing the gene expression profiles of CTCs isolated from a mouse model of pancreatic cancer
25	SVM model was used to classify new droplet digital PCR data derived from patient CTCs	Analysis of prostate CTCs using microfluidic cell enrichment followed by digital quantitation of prostate-derived transcripts
20	Whole genome sequencing of CTCs. Quantitative analysis of somatic mutations, and allele frequency evaluation	Isolation and analysis of CTCs at genomic level
24	Identification of somatic point mutations, detecting variants in scRNA-seq and improve the sensitivity and accuracy of the detected variants	Analysis of the genomic variation in CTCs in the peripheral blood of solid tumors
21	Quality assessment, differential methylation analysis, enrichment analysis, gene ontology network analysis, gene co-expression analysis, and survival analysis	The role of DNA methylation changes in CTC clusters in promoting metastasis in breast cancer patients

Despite being a useful tool, liquid biopsies suffer from a small amount of biological material. The low abundance of biological material often results in a limited pool of target biomolecules, compromising the detection sensitivity, especially for genomic variations that occur at a low frequency. This challenge often limits the utility of

liquid biopsies in early disease detection and monitoring, where the concentration of tumor-derived materials is usually very low. Thus, improving the sensitivity and specificity of the methods used for the detection and analysis of liquid biopsy samples remains a crucial task for bioinformatics research.

The integration of bioinformatics methodologies in the analysis of CTCs offers significant promise for advancing the fields of cancer research, diagnosis and treatment. One possible way to improve the interpretive power of liquid biopsies is through the integration of different omics data [36]. This multi-omics approach can potentially enhance our understanding of the intricate tumor biology, and aid in the discovery of new biomarkers or therapeutic targets. While bioinformatics methods have a transformative effect in the field of liquid biopsy, there are still numerous challenges that need to be addressed. Future research needs to focus on developing more sensitive and specific methods for the analysis of low-abundance molecules, as well as optimizing techniques for integrating and interpreting different types of omics data from liquid biopsies.

In conclusion, the integration of bioinformatics methodologies in the analysis of CTCs offers significant promise for advancing the fields of cancer research, diagnosis, and treatment.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

VU developed the concept and drafted the paper; LTDC reviewed and provided critical revisions; HS supervised the research; all authors have revised and approved the final version of the article.

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