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Early Detection of Pancreatic and Colorectal Cancers via Ultra-Sensitive Circulating Tumor DNA (ctDNA) Analysis

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Abstract

Background and Objectives: Pancreatic and colorectal cancers are frequently diagnosed at advanced stages, significantly limiting treatment options and reducing survival rates. To address this critical challenge, non-invasive early detection methods that leverage circulating tumor DNA (ctDNA) for identifying tumor-specific genetic mutations in plasma have been proposed. ctDNA analysis provides a high-precision, dynamic approach for detecting molecular signatures of cancer at earlier stages compared to traditional diagnostic methods such as endoscopy, imaging, or biopsy, which are often limited to identifying tumors in advanced stages. **Methods**: This review examines current research on next-generation sequencing (NGS) and highly sensitive digital PCR techniques for detecting minimal amounts of ctDNA shed by cancer cells into the bloodstream. By evaluating the technological advancements and methodologies used for ctDNA analysis, this review explores the potential of these approaches for early detection, continuous monitoring of ctDNA fluctuations, and real-time assessment of tumor progression or therapy response. **Results**: The reviewed studies demonstrate the capacity of ctDNA analysis for early cancer detection and personalized disease monitoring. Its ability to provide dynamic, molecular-level insights makes it particularly valuable for high-risk populations with genetic predispositions to pancreatic and colorectal cancers. However, challenges remain, including enhancing the sensitivity and specificity of ctDNA detection to accurately differentiate between benign and malignant alterations. **Conclusions**: ctDNA-based early detection has the potential

to revolutionize cancer screening and patient management, offering a personalized and non-invasive approach to identifying and monitoring pancreatic and colorectal cancers. Further research is necessary to optimize detection technologies, validate their effectiveness across diverse populations, and integrate these methods into clinical practice. This innovative strategy holds promise for improving early diagnosis and therapeutic outcomes, ultimately transforming the landscape of cancer care.

Keywords

Circulating Tumor DNA (ctDNA), Next-Generation Sequencing (NGS), Pancreatic Cancer, Colorectal Cancer, Early Detection, Precision Medicine

1. Introduction

Pancreatic and colorectal cancers rank among the leading causes of cancer-related deaths in the United States. The incidence of pancreatic cancer increases with age and is more prevalent in men than women [1], while colorectal cancer rates have risen globally for both sexes [2]. Annually, pancreatic cancer causes over 300,000 deaths, and colorectal cancer accounts for more than 600,000 deaths worldwide [3]. Both cancers present major public health challenges due to their frequent late-stage diagnosis and high mortality rates. Despite advancements in diagnostic techniques, many cases are not detected until they reach advanced stages. While diagnostic markers can aid in early detection and extend overall survival [4], methods like colonoscopy, which is considered the gold standard for colorectal cancer screening, are expensive and face issues with patient compliance, limiting their effectiveness. Stool-based tests are another popular method for colorectal cancer detection, praised for their low cost and feasibility as at-home tests. Despite these advantages, this screening method still faces limitations that muddle its effectiveness, such as low specificity and sensitivity and certain food-based substances causing false positives [3]. Similarly, pancreatic cancer detection faces shortcomings as there are no standard methods for screening or early detection [1]. Although developments have been made in diagnostic techniques through the years, due to variability and significant limitations, these cancers are still detected at advanced stages, decreasing their chances of successful treatment. As incidence and mortality rates continue to rise, novel approaches are urgently needed to improve patient outcomes.

Early cancer detection enhances treatment success, increasing the chances of cure or prolonged survival. In colorectal cancer, prognosis is closely tied to the timing and stage of diagnosis [3], underscoring the critical role of early screening. Detecting cancer early greatly increases the likelihood of successful treatment, offering the potential for a cure or longer survival. As cancer incidence and mortality rates rise, innovative screening methods are becoming more essential to improve patient outcomes. Circulating tumor DNA (ctDNA) is emerging as a prom-

ising biomarker for early cancer detection.

Tissue biopsy is currently the gold standard for tumor identification, but it has several drawbacks, including its invasive nature and often low patient compliance. Additionally, it struggles to detect tumor heterogeneity, which complicates accurate tumor identification [5]. Liquid biopsies, on the other hand, provide a less invasive approach by detecting tumor biomarkers through the analysis of circulating tumor DNA (ctDNA). Liquid biopsy technology offers a more effective way to address tumor heterogeneity and allows for repeated testing. It enables comprehensive, real-time molecular monitoring, providing insights into tumor burden and genetic changes throughout the entire course of the disease [5]. ctDNA has already made unique contributions to the field of oncology. One study showed that ctDNA successfully identified KRAS mutations with relatively 99% specificity in a group of patients with colorectal cancer [4]. Another study followed ctDNA in the colorectal cancer tumors of several patients and identified specific genes with frequent mutations, including APC, KRAS, and TP53. In recent years, next-generation sequencing (NGS) has gained popularity for detecting ctDNA. A team of researchers developed an NGS-based ctDNA assay to determine its sensitivity and specificity compared to ddPCR, which is more commonly used. It was found that the NGS-based ctDNA assay was able to detect more mutations in disease progression compared to ddPCR [5]. Due to its minimally invasive nature and ability to allow frequent monitoring, ctDNA analysis could refashion cancer screening by improving early detection, tracking disease progression, and personalizing treatment approaches.

2. Circulating Tumor DNA (ctDNA) and Cancer Detection

2.1. Definition and Biological Origin of ctDNA

Circulating tumor DNA (ctDNA) refers to small DNA fragments released into the bloodstream by tumor cells. These DNA fragments are often shed due to tumor cell apoptosis, necrosis, or active secretion during cellular turnover [6]. ctDNA represents a subset of cell-free DNA (cfDNA) found in the plasma of healthy individuals and patients with various diseases, including cancer. The key difference between ctDNA and other cfDNA is the presence of tumor-specific genetic alterations, such as mutations, methylation patterns, and copy number variations, that reflect the genetic landscape of the tumor from which the DNA originates [8]. Because ctDNA is derived directly from tumors, it provides a non-invasive means to study the genomic alterations in cancer, making it an ideal biomarker for cancer diagnosis, prognosis, and monitoring.

2.2. Advantages of ctDNA Analysis over Traditional Diagnostic Methods

The traditional methods of cancer diagnosis-biopsy, imaging, and endoscopy are often invasive, time-consuming, and associated with risks such as infection and bleeding [7]. In contrast, ctDNA analysis offers a non-invasive and highly sensi-

tive method for detecting cancer-associated genetic mutations, providing a sense of relief from the burden of invasive procedures. It enables the detection of cancers at earlier stages compared to traditional diagnostics, which are often limited to identifying tumors once they have become sizable enough to be visible on imaging or to cause clinical symptoms. The sensitivity of ctDNA analysis has been demonstrated through next-generation sequencing (NGS) and digital polymerase chain reaction (dPCR), which can identify low levels of ctDNA in the bloodstream, corresponding to early-stage tumors [9]. Moreover, ctDNA can provide a dynamic picture of the tumor's molecular evolution, allowing clinicians to monitor tumor progression or treatment response in real-time. This is particularly advantageous in the management of cancers like pancreatic and colorectal, where early detection is crucial for improving survival rates [10].

2.3. Current Applications of ctDNA in Cancer Management

ctDNA analysis has already found applications in several aspects of cancer management, including early detection, treatment response monitoring, and minimal residual disease (MRD) detection. In early detection, ctDNA screening can identify tumor-specific mutations in asymptomatic individuals, particularly those at high risk for cancer due to genetic predispositions. For example, studies have shown that ctDNA can detect early-stage pancreatic and colorectal cancers before clinical symptoms manifest, significantly improving outcomes [11]. Furthermore, ctDNA is increasingly used to monitor therapeutic efficacy. During cancer treatment, ctDNA levels can be measured to evaluate whether a patient is responding to therapy or if there is evidence of resistance or disease progression [12]. The ability of ctDNA to detect MRD after surgery or chemotherapy also provides valuable information about the likelihood of cancer recurrence, allowing for earlier interventions if needed.

2.4. Challenges in ctDNA Detection and Analysis

While ctDNA holds great promise, several challenges remain in its detection and analysis. One major challenge is the relatively low abundance of ctDNA, especially in early-stage cancers, where the amount of tumor DNA circulating in the blood can be minimal. This makes it difficult to differentiate ctDNA from the background cfDNA originating from normal cells [7]. To overcome this, highly sensitive techniques such as NGS and digital PCR have been developed, but these methods can be costly and require specialized equipment, limiting their widespread use [13]. Additionally, ctDNA analysis faces challenges in terms of specificity. Tumor-specific mutations must be carefully differentiated from benign alterations in cfDNA to avoid false positives. Moreover, ctDNA only provides a snapshot of the tumor's genetic profile during blood sampling, which may not fully capture the tumor's heterogeneity [14]. As such, while ctDNA represents an exciting frontier in cancer diagnostics, further research is necessary to refine the technology and address these limitations.

Circulating tumor DNA has emerged as a promising tool for the early detection and management of cancer, offering a non-invasive, highly sensitive method to monitor tumor-specific genetic alterations. Its use in screening, assessing treatment response, and detecting minimal residual disease highlights its potential to revolutionize cancer care, particularly in hard-to-detect malignancies like pancreatic and colorectal cancers. However, challenges such as low ctDNA abundance and specificity must be addressed before ctDNA can become a routine part of clinical practice. Future advancements in detection technologies and further research into ctDNA biology are necessary to realize its full potential.

2.5. Mechanisms of ctDNA Release and Detection

Circulating tumor DNA (ctDNA) refers to small fragments of DNA carried by cancer cells into the bloodstream. The mechanisms of ctDNA secretion are complex and multifaceted and are mainly concerned with biological processes in tumor cells. The best-known mechanism of release from ctDNA is apoptosis, a form of programmed cell death. During apoptosis, cell membrane disruption occurs, allowing cellular material, including fragmented DNA, to enter the bloodstream [15]. Necrosis in rapidly spreading tumors with inadequate blood flow is another major source of ctDNA release. In contrast to apoptosis, necrosis causes abrupt rupture of the cell membrane, allowing free diffusion of large DNA fragments [16].

Furthermore, actively proliferating tumor cells can shed ctDNA through mechanisms such as exosome release, where DNA is present in cell membranes and transported across the communication system. ctDNA shedding occurs in all tumor types, and various biological factors influence this phenomenon, including tumor size, lymphatic infiltration, and cell turnover rate. Larger tumors with more lymph nodes or more cellular turnover shed more ctDNA. In contrast, tumors that fail to enter the circulation may express small amounts of ctDNA, making detection difficult [17].

Minimal residual disease (MRD) is the presence of a small number of cancer cells in the body after therapy, which might eventually lead to disease recurrence. The ability to identify MRD with ctDNA offers a non-invasive and extremely sensitive method for monitoring therapy response and predicting relapse. Several factors influence ctDNA levels in MRD detection, such as tumor load, vascular proximity, and clearance rates. Tumors with enhanced neovascularization, such as colorectal cancer, have higher ctDNA levels, making them more suitable for MRD monitoring [18].

The half-life of ctDNA in blood is short, typically a few hours, due to rapid clearance by the liver and kidneys. As ctDNA is only present in minimal amounts in MRD conditions, high-sensitivity detection techniques are required. CtDNA levels can also be influenced by patient-specific variables, such as immunological response and genetic predisposition. For instance, elevated ctDNA levels may result from increased apoptosis or necrosis caused by heightened immunological

activity, but interpretation may be complicated by background DNA from non-cancerous cells [6].

Next-generation sequencing (NGS), which enables precise identification of tumor-specific mutations, has revolutionized MRD detection. By deep-sequencing millions of DNA fragments, NGS finds low-frequency alterations that point to a specific disease. A few examples of the various genomic changes found in NGS include point mutations, insertions, deletions, and structural rearrangements, and these are essential for evaluating MRD [10]. When identifying uncommon tumor-specific mutations in patients with low ctDNA levels, high-depth sequencing is especially valuable.

Despite its advantages, NGS faces challenges in distinguishing tumor-derived ctDNA from background noise, particularly in MRD settings where ctDNA levels are extremely low. Advanced bioinformatics tools help analyze sequencing data, filter out errors, and ensure accurate mutation detection. NGS technology is an extremely potent tool for MRD monitoring because of its constant improvements in sensitivity, specificity, and cost-effectiveness.

Digital PCR (dPCR) has emerged as an alternative, highly sensitive method for ctDNA-based MRD detection, offering unparalleled accuracy on low volumes of ctDNA. Each fragment contains zero or one ctDNA molecule, allowing rare mutations to be detected and quantified at the single-molecule level. This extreme sensitivity makes dPCR particularly useful for monitoring ctDNA in early cancer or relatively residual disease after treatment [5].

The main advantage of dPCR over NGS is its ability to detect highly sensitive mutations with very low background noise. However, dPCR has a limited ability to detect unknown mutations, making it best suited for following known oncogenic drivers. Combining NGS and dPCR may provide a comprehensive strategy for MRD detection, using NGS' broad mutation detection capability and dPCR's high sensitivity [5].

A major challenge in ctDNA-based MRD analysis is distinguishing true cancer-associated mutations from benign mutations. Some genetic alterations detected in ctDNA may be related to clonal hematopoiesis, an age-related process in which mutations accumulate in hematopoietic stem cells rather than tumor cells [19]. Advanced bioinformatics techniques are essential for distinguishing tumor-specific mutations from these non-cancerous variants to increase specificity.

ctDNA sequences can be compared with known mutation databases, such as COSMIC (Catalogue of Somatic Mutations In Cancer), to identify mutations commonly associated with specific cancer types [20]. By integrating these findings with clinical and molecular data, ctDNA-based MRD testing can confirm tumor origins and improve its specificity, reducing the chances of false positives [21].

As ctDNA detection methods advance, attempts are underway to standardize methods for MRD assessment. Setting standardized protocols for sample collection, processing, and analysis can increase repeatability and ease clinical implementation. Future research should focus on optimizing ctDNA-based MRD de-

tection to allow for earlier cancer relapse prediction and individualized treatment changes, ultimately improving patient outcomes [22].

3. Current Evidence from Research

ctDNA detection studies in pancreatic and colorectal cancers (CRC) have increased due to their non-invasive nature and ability to provide comprehensive information that can help guide treatment options. Historically, pancreatic cancer has molecular drivers such as KRAS, TP53, SMAD4, and CDKN2A [23]. Utilizing ctDNA, researchers are afforded an alternative approach to determine the heterogeneity of tumor cells compared to conventional biopsy. Many studies have found that the level of ctDNA increases in relation to disease progression. In pancreatic cancer specifically, tumor volume correlated with the amount of ctDNA.

One study conducted in 2020 found ctDNA in pancreatic cancer to be detected in 48% of localized tumors, but in more than 80% of advanced cancers [24]. Several studies question the utility of ctDNA in early-stage pancreatic cancer [24]. The mechanism of release of ctDNA is still being postulated and dependent on numerous factors such as cancer staging. A key advantage of ctDNA is that it is absent in healthy cells, allowing researchers to distinguish between cancerous and non-cancerous cells. This is in contrast to CA-19-9 levels, which have been utilized to monitor response to chemotherapy response but are also found in other disease pathologies such as bile duct obstruction. A preliminary study investigated if ctDNA correlates with response to chemotherapy and found the presence of ctDNA correlated with CT-detected tumor response in 76.9% of patients [25]. In this study, 60 genes in cfDNA were screened from ten patients with metastatic pancreatic cancer. They additionally used ddPCR to identify mutations amongst a cohort of 188 patients with metastatic pancreatic cancer. Amongst the 10 patients, 6 patients had the presence of ctDNA correlating with tumor response. Baseline characteristics of this study included a total of 188 plasma samples from April 2012- August 2014. Additional data points were collected at more regular intervals of 8 or more weeks from the 13 patients mentioned above. This study by Cheng, Liu, Jiang *et al.* [25] utilized a large sample size providing a substantial dataset for studying ctDNA mutations in metastatic pancreatic patients. They also employed different methodologies both using ctDNA and ddPCR, which provide a more comprehensive mutation detection assay. Unique insights were also discovered in this study, including the ERBB2 exon 17 and KRASG12V mutations, which are pivotal in addressing targeted therapies for pancreatic cancer patients. A weakness in this study can be that although they had a large sample size of 188 patients to evaluate mutations, only a small subset of 13 patients had prospective analysis done, which may not be significant enough retrospectively. Additional weaknesses can be the single-center nature of the study and the limited reliability of the retrospective design. Botta *et al.*, in their study conducted in 2024, found ctDNA to be prognostic of recurrence and may be used for improved patient risk stratification during perioperative therapy. This study emphasizes the utility of

ctDNA as a means to achieve more personalized medicine [26]. The study design is a retrospective study that analyzes multiple cohorts of patients with pancreatic ductal adenocarcinoma stratified based on disease stage, treatment, and various time points of ctDNA testing. The various cohorts consisted of preoperative, post-operative, surveillance, and treatment-specific, e.g., neoadjuvant patients or patients having ctDNA drawn prior to treatment as opposed to after surgery. This study had 298 patients who had confirmed PDAC and 1329 plasma samples. This sample size is large enough to yield greater statistical power. Depending on the cohort of the patient, there was a longitudinal approach rather than a retrospective design like many of the studies referenced in this paper, which allows for a more comprehensive assessment of the results along with decreased chance of bias. The study also controlled for confounding factors such as KRAS mutations, which yielded increased validity. One weakness of the study is the increased stratification and heterogeneity of timing of ctDNA; this can make it difficult to draw conclusions across different groups being studied.

Colorectal cancer has distinct biomarkers compared to pancreatic cancer, such as the HERC2 gene. While both ctDNA can be used to analyze the tumor heterogeneity, the molecular profile of these cancers can vary, and there are timelines for when to collect them. In several studies conducted on ctDNA and colorectal cancer, it was found that peritoneal fluid had increased mutations over plasma, which was a preferred method of collection. Similarly to pancreatic cancer, patients can be stratified depending on ctDNA status. One instance of this can be seen in the decision for adjuvant therapy in colorectal cancer [27]. The decision to add adjuvant therapy in stage 2 colorectal cancer patients was guided by ctDNA in a study that provides information about the extent of disease and risk of recurrence after curative intent surgery [28]. Many studies emphasize the importance of ctDNA in colorectal cancer, particularly in the adjuvant setting, where it can influence decisions on post-surgical chemotherapy. In colon cancer, particularly the MRD (minimal residual disease) can be predicted utilizing ctDNA. Multicenter studies such as COBRA and CIRCULATE underscore the importance of collecting ctDNA to guide the management of patients with CRC and whether ctDNA should be implemented in standard clinical practice [28]. In pancreatic cancer, the multicenter study DYNAMIC pancreas is an ongoing study to determine the utility of ctDNA in administering adjuvant therapy for early-stage pancreatic cancer. Similarly to CA-19 and early detection of pancreatic cancer, ctDNA can be used to detect early stage and recurrence of colorectal cancer. In colorectal cancer, CEA (carcinoembryonic antigen) is one of the biomarkers that can be used to detect recurrence similar to the role CA-19-9 has in pancreatic cancer.

The sensitivity and specificity of ctDNA varies depending on the cancer type. There are also various ways of collecting ctDNA, which can determine the specificity and sensitivity of the assay. In colorectal cancer, there is seen to be a high rate of shedding of circulating tumor fragments, which makes ctDNA collection more suitable for analyzing MDB (minimal disease burden) and other character-

istics of the tumor genomic profile. In colorectal cancer ctDNA collection is seen to have a high sensitivity. The BLUE-C prospective study found a high specificity for ctDNA in CRC [28].

In pancreatic cancer, the sensitivity and specificity appear to be lower based on a meta-analysis of various studies. Multiple studies have investigated ctDNA as a screening tool compared to conventional methods such as EUS (endoscopic ultrasound) ctDNA has a sensitivity of 65% and specificity of 75% compared to the 79% sensitivity and specificity of 93% for CA 19-9 [29]. This lower sensitivity and specificity of ctDNA in the detection of pancreatic ductal adenocarcinoma can be attributed to decreased shedding and apoptosis of the pancreatic tumor cells in the early stages of the disease. Lung and breast cancers have lower ctDNA sensitivity and specificity compared to colorectal and pancreatic cancers.

ctDNA has been an ongoing focus of study for researchers in the oncology community as it can replace or supplement more invasive procedures such as biopsy. Research is still being done on which cancers are more sensitive to the collection of ctDNA and the time points that can provide optimal results. The consensus as of the research now is that it has not replaced methods such as biopsy but acts as a supplement to the methods we have in place of gathering tumor genomic information. ctDNA is limited in that it only provides information at a certain point in time, which is contingent on whether the tumor cells are being shed. Oftentimes, biopsies from CRC and pancreatic ductal adenocarcinoma can have insufficient tumor content, which makes using the less invasive technique of collecting ctDNA more appealing.

4. Proposed Ultra-Sensitive ctDNA Analysis Method

4.1. Technological Advancements

Technological advancements in cfDNA detection over the past few decades have opened the door to improved methods of detection and screening of cancer, including colorectal and pancreatic cancers. The proposed methodology for this study would integrate novel, ultra-sensitive ctDNA detection techniques such as dPCR and Next-Gen Sequencing to provide a high degree of sensitivity and accuracy.

Cell-free DNA (cfDNA) refers to the short, double-stranded DNA sequences released from apoptotic cells. It can be detected and analyzed to help create diagnostic biomarkers for a variety of diseases and physiological states, but it is of particular interest in the early detection of cancer. CfDNA can be further classified into circulating tumor DNA, or ctDNA, which is characterized as being tumor-derived and a useful biomarker for a variety of tumor-specific mutations implicated in various cancers [30].

Currently, a variety of challenges impede the utilization of ctDNA including the short length of molecules, the relatively low (and variable) concentrations, and the influence of host wellness, immune function, and infection status on real-time cfDNA concentration. These characteristics make traditional nucleic acid qPCR

testing inadequate for analyzing cfDNA clinically. Currently, digital PCR (dPCR) and Next-Gen Sequencing (NGS) are the most accurate methods for the analysis of cfDNA [30].

dPCR, also known as ddPCR (double-droplet PCR), is a method that partitions a DNA sample into microscopic, nanoliter-sized droplets that each undergoes PCR [31]. This methodology provides absolute quantitation of DNA targets. While currently slower and more expensive than qPCR, dPCR is much more accurate, sensitive, and robust when analyzing for very minor differences in sequence or when utilizing low-concentration DNA. Currently, it can detect ctDNA with mutant allele frequencies as low as 0.001% [32]. This makes it ideal for the analysis of cfDNA/ctDNA in screening for colorectal and pancreatic cancers, where early target mutations are rare and in very small concentrations.

NGS is another method commonly used to detect ctDNA mutations. It is a high-throughput method that can screen for a broad range of mutations, detecting de-novo variants [32]. However, it does have a lower sensitivity for detecting lower-frequency mutations due to background noise. Because of its broad reach, NGS is associated with higher costs as well as higher processing times compared to dPCR [33].

To improve the sensitivity of ctDNA analysis, the analytical techniques of NGS and dPCR can be integrated, utilizing the strengths of each technology. NGS serves as an ideal initial screening tool because of its capability to detect a wide range of alterations across many genes. It can be used first to provide a comprehensive initial view of the tumor DNA, and targeted NGS panels can be used to screen patients' blood samples. Once specific mutations are identified through NGS, highly sensitive dPCR methods can then be used for validation and precision quantification of these mutations. Based on NGS results, custom dPCR probes can be designed to quantify and monitor relevant cancer mutations in a patient, following them throughout treatment and monitoring for early signs of cancer recurrence. This combination of techniques is preferable as it allows the quick, broad mutation detection of NGS to be complemented by the precise, sensitive quantification achieved with dPCR. By applying these non-invasive techniques sequentially, clinicians can effectively screen for rare and aggressive cancers, track tumor dynamics, validate treatment responses, and monitor recovery with greater accuracy and less invasiveness than current protocols.

4.2. Study Design and Methodology

ctDNA is found to have a relatively low stability, which can be limited when the collection process is conducted. This technical challenge has led to innovative approaches in studies such as the creation of blood collection tubes (cfDNA Streck tubes TM), which can maintain genomic DNA stability for 14 days post-collection [34].

Given the low stability of cfDNA, a number of proposed protocols for ctDNA isolation and analysis have been proposed. cfDNA can be collected from a variety

of sources including plasma, serum, peritoneal fluid, urine, saliva, etc. The sample is processed utilizing centrifugation. The sample ctDNA can be extracted manually or automatically using columns or magnetic beads. After the extraction of ctDNA, analysis can be done using targeted sequencing methods. In the case of pancreatic cancer, these mutations are commonly KRAS, TP53, and vary depending on the tumor type we are analyzing [35]. Next-generation sequencing (NGS) allows the sequencing of large segments of ctDNA. Another method of analyzing ctDNA is ddPCR (droplet digital PCR). Droplets in ddpCR allow the DNA sequence to be separated into multiple droplets and then analyzed with PCR amplification [36].

Low-frequency mutations are critical in pancreatic and colorectal cancers, allowing for earlier detection. Traditional techniques have been insufficient in detecting low-frequency mutations. Certain techniques can include novel technology such as CRISPincette, which was designed to efficiently detect mutant alleles by eliminating abundant wild-type alleles from normal cell-induced cell-free DNA (cfDNA) using CRISPR/Cas9 [37]. This technology has still yet to be elucidated, but by eliminating background noise of normal cell ctDNA and increasing loci of the low-frequency mutations, researchers are able to further analyze the low-frequency mutations that can be present in pancreatic and colorectal cancers.

4.3. Clinical Applications

In terms of clinical applications, the detection of ctDNA shows promise for the early detection of colorectal cancers. In a 2020 study comparing the cell-free DNA (cfDNA) of patients with colorectal cancer and healthy controls, a computer algorithm trained on colorectal cancer-specific methylation markers was able to distinguish between the two groups with high accuracy. The same study found that in high-risk populations that the presence of the ctDNA methylation marker cg10673833 could detect cancerous and precancerous colorectal lesions with a sensitivity and specificity of 89.7% and 86.8%, respectively [38]. ctDNA can also be highly beneficial in the early detection of pancreatic cancer. Pancreatic cancer notoriously presents insidiously and is often already malignant or inoperable once discovered. The current standard for non-invasive screening, CA19-9, has limited use due to its low sensitivity [39]. Screening ctDNA could offer a more sensitive, non-invasive screening method for those in high-risk populations.

Additionally, continuous monitoring of ctDNA fluctuations can be used clinically to assess tumor progression and treatment response. ctDNA has a short half-life of between two and four hours, making it a changing marker of tumor burden. This can make it useful in assessing real-time response to chemotherapy and detecting minimal residual disease (MRD) post-surgery [40]. Changes in allele frequency can be early predictors of therapy response or resistance [41]. There are a multitude of studies showing the presence of ctDNA both post-chemotherapy and post-operatively decreases the length of recurrence-free survival. One Swedish study of 58 patients found that those with positive levels of post-operative ctDNA had a 77% re-

currence while all 45 ctDNA-negative patients were relapse-free [42]. These findings suggest that monitoring ctDNA levels could benefit clinicians when making decisions on the efficacy of treatment and the need for further intervention in patients.

4.4. Gaps in Knowledge and Future Research Directions

Gaps in the literature and limitations in current research across numerous studies highlight the need for further investigation. While existing findings are promising, several issues remain that underscore the necessity for more thorough and robust studies to bridge these gaps. Larger, more diverse cohort studies are needed to validate ctDNA-based early detection methods, as many current studies have small sample sizes and come from single institutions [42]. This presents challenges when generalizing results due to the lack of diversity and limited population scope. Additionally, there is minimal standardization in how the ctDNA is being collected and the assays used in its analysis. Implementing standardizations of sample collections, storage, and ctDNA extraction as well as for the assays being used in analysis would allow for pooled data analysis and meta-analyses and aid in establishing the clinical validity of ctDNA use [41]. Given the short half-life of ctDNA, the time points of sample collection post-treatment should also be standardized across studies [40] [41].

Further gaps in knowledge remain regarding the development of standardized protocols for ctDNA analysis in clinical settings, particularly in managing patients who have completed therapy without recurrence but still exhibit detectable ctDNA levels. Future research should explore how ctDNA detection in these cases can guide clinical decisions and whether adjuvant chemotherapy is warranted based on ctDNA results [42]. More studies are needed to establish clear guidelines for using ctDNA in post-treatment surveillance and treatment planning.

Another significant gap in knowledge exists regarding the ability to distinguish between benign and malignant genetic alterations, particularly in the context of clonal hematopoiesis of indeterminate potential (CHIP). CHIP involves abnormalities in the DNA of hematopoietic stem cells, which can be detected when DNA fragments from non-malignant cells are released; these fragments can lead to potential false positives in ctDNA testing [41]. These alterations become more common with age, affecting up to 10% of patients over 70 [43]. Mutations in genes like *KRAS* and *TP53*, which are implicated in both hematologic and solid malignancies, are particularly concerning compared to mutations that are unique to hematologic malignancies alone [41]. Emerging research has identified high rates of CHIP in patients with solid tumors, driven in part by oncologic therapy [43]. Thus, before using ctDNA to guide clinical decisions post-chemotherapy, future research is needed to refine methods for accurately differentiating CHIP-related mutations from true malignancies both to improve diagnostic precision and to reduce unnecessary interventions. Standardizing detection protocols and incorporating age-related factors into genetic analysis could further enhance the accuracy of these distinctions.

4.5. Implications for Clinical Practice and Public Health

ctDNA analysis holds great promise for the early detection of colorectal and pancreatic cancer screening. Early detection is a key consideration, given that these cancers are often found as malignancies with limited available treatment options [7]. Since early detection is the most optimal time for treatment, ctDNA screening serves as a useful technique for increasing survival [6]. High-risk individuals with a genetic predisposition or family history of cancer benefit most from this minimally invasive procedure, which is performed using a routine blood test [43] By identifying cancer before symptoms appear, ctDNA testing can further reduce the time needed to plan a salvage treatment [44].

Despite its promising clinical value, ctDNA-based testing is also subject to limitations. Its applicability and sensitivity, particularly for cancers in the early stages, are perhaps the most important limitations. False positives can lead to unjustified interventions, while false negatives can lead to delayed diagnosis [45]. In addition to this, inter-patient and inter-tumor variations in ctDNA levels make test reproducibility and standardization challenging. To overcome such challenges, additional studies and larger clinical trials need to be performed to demonstrate the consistency of ctDNA across various populations and cancer types.

Cost-effectiveness is another major factor in expanding the use of ctDNA-based diagnostics. Despite the potentially high initial cost, early diagnosis may save money in the long run by reducing the need for costly late-stage cancer treatments and extended palliative care. Economic models will need to quantify these savings, particularly in high-risk individuals, to assess the feasibility of routine use of ctDNA testing in clinical practice. Additionally, a comparison with available screening technologies, e.g., colonoscopy for colorectal cancer or imaging for pancreatic cancer, must be drawn to determine whether ctDNA testing is less expensive [43].

Ethical issues must also be addressed to ensure the correct use of ctDNA-based testing as ctDNA analysis involves working with sensitive genetic material. Therefore, strict measures must be implemented to protect patient privacy and prevent illegal access by employers or insurance providers [46]. Furthermore, disparities in access to ctDNA testing must be minimized to ensure equitable provision of healthcare. Policymakers must look into measures such as the extension of insurance coverage and subsidized testing initiatives to prevent socioeconomic obstacles from hindering patient access.

The clinical efficacy of ctDNA-based testing must be carefully evaluated against issues of cost, ethics, and accessibility. In order to maximize the benefits of ctDNA testing while upholding strict patient care and confidentiality requirements, these issues must be resolved through comprehensive validation studies, legislative measures, and justifiable implementation strategies [6].

5. Conclusions

Pancreatic and colorectal cancers are current leading causes of cancer-related death in the US. Modern gold-standard screening methods such as colonoscopy

or tissue biopsy are expensive, time-consuming, and difficult to access, contributing to the frequent late-stage diagnosis and high mortality rates in these cancers. This matter has become more urgent in recent years as the diagnosis rates increase, and the age at diagnosis decreases. Together, these factors point toward the urgent need for innovative, effective and non-invasive screening tools.

ctDNA is a groundbreaking tool in cancer care, providing a non-invasive and highly sensitive method for early detection, monitoring, and management of difficult-to-treat malignancies such as pancreatic and colorectal cancer. By providing real-time insights into tumor biology, ctDNA is a valuable alternative to traditional diagnostic methods such as tissue biopsies, imaging, and serum markers. Digital PCR (dPCR) and next-generation sequencing (NGS) are unique techniques for ctDNA analysis. dPCR excels in precision and sensitivity, especially for detecting low-frequency mutations. NGS is better suited for broad mutation screening across multiple genes. A combined approach with these technologies could enhance ctDNA analysis.

Future directions in ctDNA research should focus on standardizing detection methods, enhancing sensitivity and specificity, and refining clinical decision-making frameworks. ctDNA has the potential to reform cancer care by offering precise, individualized, and less invasive diagnostic and therapeutic strategies. With continued advancements, ctDNA-based testing could significantly improve survival rates and quality of life for cancer patients.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Ilic, M. and Ilic, I. (2016) Epidemiology of Pancreatic Cancer. *World Journal of Gastroenterology*, **22**, 9694-9705. https://doi.org/10.3748/wjg.v22.i44.9694

[2] Akimoto, N., Ugai, T., Zhong, R., Hamada, T., Fujiyoshi, K., Giannakis, M., *et al.* (2020) Rising Incidence of Early-Onset Colorectal Cancer—A Call to Action. *Nature Reviews Clinical Oncology*, **18**, 230-243. https://doi.org/10.1038/s41571-020-00445-1

[3] Zhang, Y., Wang, Y., Zhang, B., Li, P. and Zhao, Y. (2023) Methods and Biomarkers for Early Detection, Prediction, and Diagnosis of Colorectal Cancer. *Biomedicine & Pharmacotherapy*, **163**, Article 114786. https://doi.org/10.1016/j.biopha.2023.114786

[4] Zygulska, A.L. and Pierzchalski, P. (2022) Novel Diagnostic Biomarkers in Colorectal Cancer. *International Journal of Molecular Sciences*, **23**, Article 852. https://doi.org/10.3390/ijms23020852

[5] Zhou, H., Zhu, L., Song, J., Wang, G., Li, P., Li, W., *et al.* (2022) Liquid Biopsy at the Frontier of Detection, Prognosis and Progression Monitoring in Colorectal Cancer. *Molecular Cancer*, **21**, Article No. 86. https://doi.org/10.1186/s12943-022-01556-2

[6] Wan, J.C.M., Massie, C., Garcia-Corbacho, J., Mouliere, F., Brenton, J.D., Caldas, C., *et al.* (2017) Liquid Biopsies Come of Age: Towards Implementation of Circulating Tumour DNA. *Nature Reviews Cancer*, **17**, 223-238. https://doi.org/10.1038/nrc.2017.7

[7] Cohen, J.D., Li, L., Wang, Y., Thoburn, C., Afsari, B., Danilova, L., *et al.* (2018) Detection and Localization of Surgically Resectable Cancers with a Multi-Analyte Blood Test. *Science*, **359**, 926-930. https://doi.org/10.1126/science.aar3247

[8] Diaz, L.A. and Bardelli, A. (2014) Liquid Biopsies: Genotyping Circulating Tumor DNA. *Journal of Clinical Oncology*, **32**, 579-586. https://doi.org/10.1200/jco.2012.45.2011

[9] Esposito, A., Criscitiello, C., Locatelli, M., Milano, M. and Curigliano, G. (2016) Liquid Biopsies for Solid Tumors: Understanding Tumor Heterogeneity and Real Time Monitoring of Early Resistance to Targeted Therapies. *Pharmacology & Therapeutics*, **157**, 120-124. https://doi.org/10.1016/j.pharmthera.2015.11.007

[10] Jiang, P., Chan, C.W.M., Chan, K.C.A., Cheng, S.H., Wong, J., Wong, V.W., *et al.* (2015) Lengthening and Shortening of Plasma DNA in Hepatocellular Carcinoma Patients. *Proceedings of the National Academy of Sciences*, **112**, E1317-E1325. https://doi.org/10.1073/pnas.1500076112

[11] Liu, M.C., Oxnard, G.R., Klein, E.A., Swanton, C., Seiden, M.V., Liu, M.C., *et al.* (2020) Sensitive and Specific Multi-Cancer Detection and Localization Using Methylation Signatures in Cell-Free DNA. *Annals of Oncology*, **31**, 745-759. https://doi.org/10.1016/j.annonc.2020.02.011

[12] Lecomte, T., Ceze, N., Dorval, É. and Laurent-Puig, P. (2010) Circulating Free Tumor DNA and Colorectal Cancer. *Gastroentérologie Clinique et Biologique*, **34**, 662-681. https://doi.org/10.1016/j.gcb.2009.04.015

[13] Sivapalan, L., Kocher, H.M., Ross-Adams, H. and Chelala, C. (2021) Molecular Profiling of ctDNA in Pancreatic Cancer: Opportunities and Challenges for Clinical Application. *Pancreatology*, **21**, 363-378. https://doi.org/10.1016/j.pan.2020.12.017

[14] Siravegna, G. and Bardelli, A. (2015) Blood Circulating Tumor DNA for Non-Invasive Genotyping of Colon Cancer Patients. *Molecular Oncology*, **10**, 475-480. https://doi.org/10.1016/j.molonc.2015.12.005

[15] Crowley, E., Di Nicolantonio, F., Loupakis, F. and Bardelli, A. (2013) Liquid Biopsy: Monitoring Cancer-Genetics in the Blood. *Nature Reviews Clinical Oncology*, **10**, 472-484. https://doi.org/10.1038/nrclinonc.2013.110

[16] Diehl, F., Schmidt, K., Choti, M.A., Romans, K., Goodman, S., Li, M., *et al.* (2008) Circulating Mutant DNA to Assess Tumor Dynamics. *Nature Medicine*, **14**, 985-990. https://doi.org/10.1038/nm.1789

[17] Schwarzenbach, H., Hoon, D.S.B. and Pantel, K. (2011) Cell-Free Nucleic Acids as Biomarkers in Cancer Patients. *Nature Reviews Cancer*, **11**, 426-437. https://doi.org/10.1038/nrc3066

[18] Bettegowda, C., Sausen, M., Leary, R.J., Kinde, I., Wang, Y., Agrawal, N., *et al.* (2014) Detection of Circulating Tumor DNA in Early- and Late-Stage Human Malignancies. *Science Translational Medicine*, **6**, 224ra24. https://doi.org/10.1126/scitranslmed.3007094

[19] Genovese, G., Kähler, A.K., Handsaker, R.E., Lindberg, J., Rose, S.A., Bakhoum, S.F., *et al.* (2014) Clonal Hematopoiesis and Blood-Cancer Risk Inferred from Blood DNA Sequence. *New England Journal of Medicine*, **371**, 2477-2487. https://doi.org/10.1056/nejmoa1409405

[20] Forbes, S.A., Beare, D., Gunasekaran, P., Leung, K., Bindal, N., Boutselakis, H., *et al.* (2014) COSMIC: Exploring the World's Knowledge of Somatic Mutations in Human Cancer. *Nucleic Acids Research*, **43**, D805-D811. https://doi.org/10.1093/nar/gku1075

[21] Murtaza, M., Dawson, S., Tsui, D.W.Y., Gale, D., Forshew, T., Piskorz, A.M., *et al.* (2013) Non-Invasive Analysis of Acquired Resistance to Cancer Therapy by Sequencing of Plasma DNA. *Nature*, **497**, 108-112. https://doi.org/10.1038/nature12065

[22] Newman, A.M., Lovejoy, A.F., Klass, D.M., Kurtz, D.M., Chabon, J.J., Scherer, F., *et al.* (2016) Integrated Digital Error Suppression for Improved Detection of Circulating Tumor DNA. *Nature Biotechnology*, **34**, 547-555. https://doi.org/10.1038/nbt.3520

[23] Watanabe, K., Nakamura, T., Kimura, Y., Motoya, M., Kojima, S., Kuraya, T., *et al.* (2022) Tumor-Informed Approach Improved ctDNA Detection Rate in Resected Pancreatic Cancer. *International Journal of Molecular Sciences*, **23**, Article 11521. https://doi.org/10.3390/ijms231911521

[24] Jaworski, J.J., Morgan, R.D. and Sivakumar, S. (2020) Circulating Cell-Free Tumour DNA for Early Detection of Pancreatic Cancer. *Cancers*, **12**, Article 3704. https://doi.org/10.3390/cancers12123704

[25] Cheng, H., Liu, C., Jiang, J., Luo, G., Lu, Y., Jin, K., *et al.* (2017) Analysis of ctDNA to Predict Prognosis and Monitor Treatment Responses in Metastatic Pancreatic Cancer Patients. *International Journal of Cancer*, **140**, 2344-2350. https://doi.org/10.1002/ijc.30650

[26] Botta, G.P., Abdelrahim, M., Drengler, R.L., Aushev, V.N., Esmail, A., Laliotis, G., *et al.* (2024) Association of Personalized and Tumor-Informed ctDNA with Patient Survival Outcomes in Pancreatic Adenocarcinoma. *The Oncologist*, **29**, 859-869. https://doi.org/10.1093/oncolo/oyae155

[27] Krell, M., Llera, B. and Brown, Z.J. (2023) Circulating Tumor DNA and Management of Colorectal Cancer. *Cancers*, **16**, Article 21. https://doi.org/10.3390/cancers16010021

[28] Malla, M., Loree, J.M., Kasi, P.M. and Parikh, A.R. (2022) Using Circulating Tumor DNA in Colorectal Cancer: Current and Evolving Practices. *Journal of Clinical Oncology*, **40**, 2846-2857. https://doi.org/10.1200/jco.21.02615

[29] Kirchweger, P., Wundsam, H.V. and Rumpold, H. (2022) Circulating Tumor DNA for Diagnosis, Prognosis and Treatment of Gastrointestinal Malignancies. *World Journal of Clinical Oncology*, **13**, 473-484. https://doi.org/10.5306/wjco.v13.i6.473

[30] Mouliere, F., Chandrananda, D., Piskorz, A.M., Moore, E.K., Morris, J., Ahlborn, L.B., *et al.* (2018). Enhanced Detection of Circulating Tumor DNA by Fragment Size Analysis. *Nature Biomedical Engineering*, **10**, eaat4921. https://doi.org/10.1126/scitranslmed.aat4921

[31] Hindson, B.J., Ness, K.D., Masquelier, D.A., Belgrader, P., Heredia, N.J., Makarewicz, A.J., *et al.* (2011) High-Throughput Droplet Digital PCR System for Absolute Quantitation of DNA Copy Number. *Analytical Chemistry*, **83**, 8604-8610. https://doi.org/10.1021/ac202028g

[32] Goodwin, S., McPherson, J.D. and McCombie, W.R. (2016) Coming of Age: Ten Years of Next-Generation Sequencing Technologies. *Nature Reviews Genetics*, **17**, 333-351. https://doi.org/10.1038/nrg.2016.49

[33] van Dijk, E.L., Auger, H., Jaszczyszyn, Y. and Thermes, C. (2014) Ten Years of Next-Generation Sequencing Technology. *Trends in Genetics*, **30**, 418-426. https://doi.org/10.1016/j.tig.2014.07.001

[34] ScienceDirect Topics (2020) Droplet Digital Polymerase Chain Reaction. https://www.sciencedirect.com/topics/medicine-and-dentistry/droplet-digital-polymerase-chain-reaction

[35] Zhang, H., Liu, R., Yan, C., Liu, L., Tong, Z., Jiang, W., *et al.* (2019) Advantage of

Next-Generation Sequencing in Dynamic Monitoring of Circulating Tumor DNA over Droplet Digital PCR in Cetuximab Treated Colorectal Cancer Patients. *Translational Oncology*, **12**, 426-431. https://doi.org/10.1016/j.tranon.2018.11.015

[36] Kim, J., Won, Y., Kang, S., Park, T., Lee, I.S., Choi, H., *et al.* (2023) Crispincette, and Detection of Low Frequency Cancer Mutations in ctDNA. *JCO Global Oncology*, **9**, 61-61. https://doi.org/10.1200/go.2023.9.supplement_1.61

[37] Luo, H., Zhao, Q., Wei, W., Zheng, L., Yi, S., Li, G., *et al.* (2020) Circulating Tumor DNA Methylation Profiles Enable Early Diagnosis, Prognosis Prediction, and Screening for Colorectal Cancer. *Science Translational Medicine*, **12**, eaax7533. https://doi.org/10.1126/scitranslmed.aax7533

[38] Yang, J., Xu, R., Wang, C., Qiu, J., Ren, B. and You, L. (2021) Early Screening and Diagnosis Strategies of Pancreatic Cancer: A Comprehensive Review. *Cancer Communications*, **41**, 1257-1274. https://doi.org/10.1002/cac2.12204

[39] Loft, M., To, Y.H., Gibbs, P. and Tie, J. (2023) Clinical Application of Circulating Tumour DNA in Colorectal Cancer. *The Lancet Gastroenterology & Hepatology*, **8**, 837-852. https://doi.org/10.1016/s2468-1253(23)00146-2

[40] Dasari, A., Morris, V.K., Allegra, C.J., Atreya, C., Benson, A.B., Boland, P., *et al.* (2020) ctDNA Applications and Integration in Colorectal Cancer: An NCI Colon and Rectal-Anal Task Forces Whitepaper. *Nature Reviews Clinical Oncology*, **17**, 757-770. https://doi.org/10.1038/s41571-020-0392-0

[41] Puccini, A., Martelli, V., Pastorino, A., Sciallero, S. and Sobrero, A. (2023) CtDNA to Guide Treatment of Colorectal Cancer: Ready for Standard of Care? *Current Treatment Options in Oncology*, **24**, 76-92. https://doi.org/10.1007/s11864-022-01048-x

[42] Reed, S.C., Croessmann, S. and Park, B.H. (2022) CHIP Happens: Clonal Hematopoiesis of Indeterminate Potential and Its Relationship to Solid Tumors. *Clinical Cancer Research*, **29**, 1403-1411. https://doi.org/10.1158/1078-0432.ccr-22-2598

[43] Phallen, J., Sausen, M., Adleff, V., Leal, A., Hruban, C., White, J., *et al.* (2017) Direct Detection of Early-Stage Cancers Using Circulating Tumor DNA. *Science Translational Medicine*, **9**, eaan2415. https://doi.org/10.1126/scitranslmed.aan2415

[44] Rolfo, C., Mack, P.C., Scagliotti, G.V., Baas, P., Barlesi, F., Bivona, T.G., *et al.* (2018) Liquid Biopsy for Advanced Non-Small Cell Lung Cancer (NSCLC): A Statement Paper from the IASLC. *Journal of Thoracic Oncology*, **13**, 1248-1268. https://doi.org/10.1016/j.jtho.2018.05.030

[45] Merker, J.D., Oxnard, G.R., Compton, C., Diehn, M., Hurley, P., Lazar, A.J., *et al.* (2018) Circulating Tumor DNA Analysis in Patients with Cancer: American Society of Clinical Oncology and College of American Pathologists Joint Review. *Journal of Clinical Oncology*, **36**, 1631-1641. https://doi.org/10.1200/jco.2017.76.8671

[46] Bombard, Y., Brothers, K.B., Fitzgerald-Butt, S., Garrison, N.A., Jamal, L., James, C.A., *et al.* (2019) The Responsibility to Recontact Research Participants after Reinterpretation of Genetic and Genomic Research Results. *The American Journal of Human Genetics*, **104**, 578-595. https://doi.org/10.1016/j.ajhg.2019.02.025