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Review

The Emerging Applications of Raman Spectroscopy in Clinical Oncology: A Narrative Review Focused on Circulating Tumor DNA Detection and Therapeutic Drug Monitoring

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Simple Summary: The analysis of the interaction between incident light and matter can reveal certain properties about the matter itself. This phenomenon can be utilised in the form of Raman spectroscopy. The application of Raman spectroscopy to biological matrices may enable both quantitative and qualitative analysis of the sample. There are many potential applications in medicine, particularly in oncology. Individualisation of care may be achieved by precisely measuring drug exposure in therapeutic drug monitoring, as well as precisely assessing treatment response by measuring changes in tumour DNA levels in the blood. The existing methods to measure these parameters are expensive, specialized and time-consuming. Raman spectroscopy may provide a more accessible and highly accurate alternative.

Abstract: Raman spectroscopy is a technique which involves quantitative and qualitative molecular analysis based on the interaction between incident light and isolation of scattered wavelengths in generating a molecular fingerprint. It has a broad array of potential scientific applications, encompassing areas as diverse as food science and forensics. However, it may also be highly useful in clinical oncology. A recent focus of research in oncology has been in achieving the individualisation of care. Two important strategies to achieve a so-called "precision oncology" approach may include the detection of circulating tumour DNA (ctDNA) in more objectively evaluating treatment response and guiding de-escalation and intensification approaches in systemic therapy and therapeutic drug monitoring (TDM). Therapeutic drug monitoring involves the quantitation of plasma drug levels in order to tailor medication dosing in optimizing outcomes. The existing approaches to characterize small molecules, such as fluorescence-based and chromatographic strategies, may be limited by high costs, long turnaround times, and bulky equipment. Surface-enhanced Raman spectroscopy (SERS) may be deployed by utilizing a handheld device, with the potential for point of care, rapid turnaround, low-cost assessment of clinically relevant parameters, and prompt implementation of attendant changes in treatment. Although there is a growing body of data supporting the implementation of TDM and evaluation of ctDNA in achieving precision medicine, the uptake of such approaches remains relatively limited outside of clinical trials. As stated, the nature of existing analytical methodologies may prove to be a significant barrier to the routine clinic-based implementation of such approaches. Therefore, we provide the existing evidence for SERS in alleviating these barriers. We also provide insights into how SERS could contribute to clinical oncology.

Keywords: Surface-enhanced Raman spectroscopy (SERS); circulating tumour DNA; therapeutic drug monitoring; clinical oncology; precision oncology

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1. Introduction

Raman spectroscopy is an analytical approach which is derived from the characterization of the interaction of incident light and matter. Through the analysis of this phenomenon, the properties of matter, both from a qualitative and quantitative perspective, can be extrapolated. The practical application of this technique in the characterization of molecules often requires the enhancement of the measured signal through utilizing inert metal surfaces, known as surface-enhanced Raman spectroscopy (SERS). This approach may be preferable to existing strategies involving techniques such as chromatography or fluoroscopy, necessitating bulky equipment, long turnaround times, and high costs. The practical utility of such approaches has been proposed in fields as diverse as food science and forensics. However, there is an increasing appreciation for the diverse potential applications in clinical medicine. More specifically, there has also been widespread interest in the applications of this technology in cancer care.

The systemic approach to the management of solid organ and hematological malignancy has been revolutionized in the era of precision medicine. For instance, individualizing treatment approaches according to specific molecular targets has proved transformative since the inception of imatinib for chronic lymphocytic leukemia. Such strategies have enabled the administration of the "right drug" to the "right patient". Another important focus of precision oncology is determining the "right dose". Therefore, dose individualization and treatment intensification and de-intensification are important goals in maximizing both the efficacy and tolerability of therapies. Two important strategies for dose individualization include the measurement of the effects or levels of administered medications or their metabolites in body fluids, or therapeutic drug monitoring (TDM), or detection of circulating tumour DNA (ctDNA), enabling exquisite assessment of treatment response and subsequent up- or down-titration of treatment intensity.

In this Opinion, we begin with the description of Raman spectroscopy and SERS, and then discuss the applications of SERS in clinical oncology, with an emphasis on the role of SERS in TDM and ctDNA detection. Lastly, we discuss the current limitations and provide our insights into the future directions of SERS in clinical oncology.

2. Description of Raman and SERS

On encountering matter, the majority of incident photons of light undergo elastic scatter, whereby the energy of the incident photons are preserved. This is known as Rayleigh scatter. However, an infinitesimal proportion of incident light (0.0000001%) undergoes inelastic scattering, in which the energy of the scattered photons change. This may be visualized as an alteration in the colour of the scattered light due to changes in wavelength. When the energy of the scattered photons is lost, this is referred to as Stokes scatter, while if a gain in energy of scattered photons occurs, this is termed anti-Stokes scatter (Figure 1). This phenomenon was described as Raman scattering and earned C.V. Raman and K.S. Krishnan the 1930 Nobel Prize in Physics [1].

The characterization of the proportion of light undergoing Raman scattering to generate information about the structural facets of specific molecules yields the practical tool of Raman spectroscopy. These facets may include the chemical structure, phase, polymorphism, and intrinsic stress/strain of the underlying molecule, as well as detection of any contaminating materials. The intensity and wavelength position of the peaks of scattered light are represented in the Raman spectrum. Therefore, a quantitative assessment corresponding to the intensity of scattered light, as well as qualitative assessment corresponding to the wavelength of each peak, may be determined from this spectrum [2]. Obviously, given the minute quantity of light undergoing this change, detection of this signal is exceedingly difficult. However, exquisite molecular characterization of tiny amounts of matter is possible through exponentially enhancing this signal utilizing roughened inert metal surfaces, mostly gold or silver, culminating in the technique of surface-enhanced Raman spectroscopy (SERS).

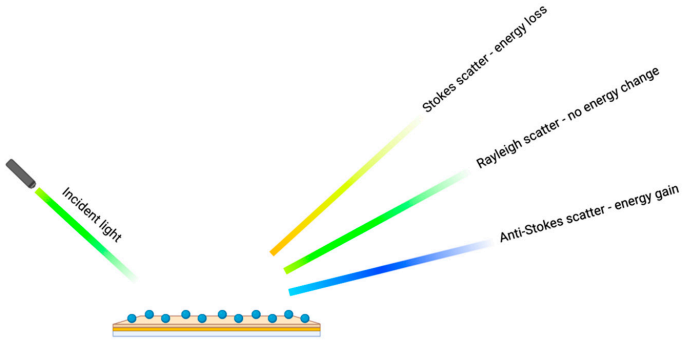

Figure 1. Phenomenon of Raman scattering as an incident light source interacts with the analyte sample.

The phenomenon of surface-enhancement in Raman spectroscopy was initially described in the 1970s, where Fleischmann and colleagues identified the exponential enhancement of the generated Raman spectrum when pyridine molecules were adsorbed onto an electrochemically roughed silver surface [2]. The resulting magnitude of enhancement in the intensity of Raman scattering has been calculated to be in the order of 10^{10} to 10^{12} for certain molecules [3]. The specific mechanisms underlying this signal augmentation is unclear, but both electromagnetic and chemical processes have been described. The electromagnetic explanation involves plasmon resonance excitation, while the chemical mechanism involves bond formation between the analyte molecules and the metal surface, leading to charge transfer and increased polarizability of the analyte molecules, culminating in amplification of the Raman signal [3,4]. A variety of inert metals may be employed for SERS, although copper, silver, and gold have most frequently been utilized [5]. Handheld devices capable of real-time quantitative and qualitative analysis of matter via SERS have been developed.

There are two separate methodologies for the practical detection of biomarkers utilizing SERS approaches: label-free or direct SERS, and label-based or indirect SERS [6]. Direct SERS approaches involve the detection of the target analyte without the incorporation of tags or probes. This method is predicated on the ability of the analyte molecule to interact with the surface plasmon and produce a corresponding SERS signal [7,8]. These strategies do offer numerous benefits, including efficiency, simplicity, and the potential to isolate a range of targets [9]. However, there are several significant limitations, perhaps most importantly being interference from other components of biological matrices. Biological matrices are exceptionally complex. Label-free SERS approaches may be confounded by interference from other molecules in the matrix, producing other signals. This interference may be addressed by utilizing statistical methods such as principal components analysis (PCA) [10], multivariate analysis [11], as well as machine learning approaches [11]. Conversely, indirect SERS involves deploying a SERS nanotag which specifically binds to the target analyte [12]. The nanotag comprises a metal nanoparticle (classically gold or silver) that augments the SERS signal of a Raman reporter molecule bound to the metal surface, complexed with a molecule (e.g., antibody) designed to bind specifically to the target analyte [13].

3. The Potential Applications of SERS in Clinical Oncology

There is an array of potential applications for Raman and SERS in clinical oncology. These include biomarker detection, histopathological analysis, surgical guidance, as well as treatment response assessment [14].

Biomarker detection and monitoring plays a critical role in the diagnostic, prognostic, and therapeutic approach employed in many cancers. Unfortunately, many commonly employed biomarkers, such as prostate-specific antigen (PSA), lack specificity when used in diagnostic settings. Due to the significant specificity of SERS-based approaches to molecular detection, biomarkers circulating at exquisitely low levels may be detectable through such strategies. Various protein biomarkers as well as microRNAs have been previously de-

tected by utilizing SERS-based strategies with an exceptionally low limit of detection (LOD), often exceeding the nanomolar level, for numerous malignancies, including prostate [15], liver [16], breast [17], and bladder cancer [18]. SERS-based approaches also offer capacity for multiplexing, enabling the simultaneous detection of protean biomarkers. With time, such approaches could potentially be utilized with machine learning approaches for exceptionally sensitive and specific detection of early cancer spectral signatures. Indeed, Dong and colleagues [19] recently presented a label-free SERS and machine learning method (SERS-AICS) for expedited cancer diagnosis. The sera of 1582 cancer patients with 5 different tumour subtypes and 382 healthy controls were analyzed using this method. Healthy controls and cancer patients were successfully distinguished with an accuracy, sensitivity, and specificity all exceeding 95%.

A significant factor complicating cancer diagnostics is accurate histopathological analysis. Although morphological assessment by a trained pathologists, with the utilization of techniques such as immunohistochemical detection of certain proteins, in addition to potentially molecular analysis, may be beneficial, oftentimes there is still uncertainty as to the specific pathological entity at play. For instance, risk stratification in localized prostate cancer and treatment planning is highly predicated on tumour differentiation, often quantified using the Gleason score. However, the inter-observer reproducibility of score determination is significant [20,21]. Similar problems for cancer grading exist for other tumours such as breast cancer [22]. SERS may offer an opportunity to achieve expedited and more accurate histopathological assessments. Spectral signatures can be derived from various normal, pre-malignant, and malignant tissues arising from certain anatomical sites. These signatures can be used to populate models, utilizing techniques such as principal component analysis (PCA) and linear discriminant analysis (LDA). Subsequently, these models can be applied to discriminate more objectively between different histopathological entities. Previously, such approaches have been used to discriminate between normal, benign, pre-malignant, and malignant pathologies across a range of solid organ malignancies, including breast [23,24], cutaneous [25], brain [26], gastrointestinal [27], and gynecological [28] sites, with excellent performance.

During diagnostic and therapeutic intervention, there is often difficulty in determining whether biopsies are taken from the most appropriate sites in diagnostic procedures, as well as whether surgical excision of cancerous lesions are complete in therapeutic interventions. Indeed, histopathological specimens from curative intent surgery performed for various cancers might reveal positive margins, necessitating further operative interventions for the patient. In breast conserving surgery performed for breast cancer, this has been reported to occur in over 20% of cases [29]. Furthermore, diagnostic biopsies are often performed only for the pathologist to conclude that the sample is inconclusive. Therefore, strategies which enable real-time and accurate characterization of suspicious lesions, as well as the status of the margins during surgical excision, would be transformative in avoiding such situations. The in vivo application of SERS-based analyses may prove to be one such solution. The in vivo incorporation of Raman spectroscopic analyses in concert with bronchoscopic [30] and gastrointestinal endoscopy [31] to characterize suspicious lesions has previously been shown to harbour promise. In addition, multimodal spectral histopathology incorporating SERS was previously shown to potentially be useful in the intra-operative determination of tumour margins in breast-conserving surgery [32].

As such, SERS-based analytic approaches may significantly improve the diagnosis and management of patients with cancer. In recent times, two areas which have garnered significant attention in oncology are the individualisation of anti-cancer treatment utilizing TDM, as well as incorporating circulating tumour DNA (ctDNA) detection in the diagnosis and management of various cancers. However, the ideal manner in which to incorporate these potentially transformative strategies requires significant further work. Given that SERS-based analytics may enable affordable, real-time, and highly accurate drug quantitation and nucleic acid detection, such approaches may ease future research initiatives in these areas.

4. The Potential Role for SERS in TDM

A number of factors may govern a given patient's drug exposure when a medication is administered. These may include age, gender, food intake, comorbidities, and genetic factors, which affect drug absorption and elimination [33]. Therefore, when the same dose of drug is given to different patients, the measured plasma drug levels may vary by up to 1000 times [34]. Dosing according to the observed drug quantitation in plasma may permit more individualized dosing, enabling the maximization of efficacy with the minimization of toxicity. The resulting practice of TDM has been utilized in clinical settings since the 1970s. TDM is routinely employed for certain classes of therapies. These may include antibiotics, antiepileptics, immunosuppressants, and anticonvulsants.

However, TDM may potentially prove practice-changing in optimizing cancer treatment. Unfortunately, the uptake in clinical oncology has remained low. Reasons for this may include a lack of clinical expertise, complicated analytical techniques and equipment, and limited data identifying target ranges for drug exposure, as well as economic and regulatory obstacles. Furthermore, logistical issues include slow turnaround and difficulties with sample collection [35]. TDM may not prove useful for all drugs. The criteria which may connote the utility of TDM include a lack of a more readily available biomarker to assess treatment response (e.g., international normalized ratio (INR) for warfarin), the availability of an established bioanalytical method, capacity for dose modulation, a demonstrable relationship between drug exposure and response, significant interpatient variability with limited intrapatient variability in exposure, and a narrow therapeutic window. Finally, the medication must be administered over a prolonged time period [36].

Conventionally, analytical approaches for TDM are founded upon immunoassays or separation techniques deployed with mass spectrometry (MS). Immunoassays may be performed routinely, as they are more readily and quickly implemented, with capacity for automation, but are often confounded by interference by drug metabolites, structurally similar exogenous and endogenous compounds, and matrix constituents. Hence, while being sensitive strategies for TDM, a lack of specificity may limit their accuracy. In addition, immunoassays involve longer reporting times (~4 h) and large, bulky equipment, hence limiting their deployment to well-funded laboratories. Given the lack of specificity affiliated with immunoassay-based approaches, liquid chromatography in concert with tandem mass spectrometry (LC-MS/MS) approaches are often considered the gold standard for TDM as they are less subject to interference. Despite their excellent analytical performance, these approaches are hampered by requirements for complex sample pretreatment and large volumes of samples required for biofluid processing. The need for this nuanced sample pretreatment necessitates trained personnel and longer turnaround times. Furthermore, similar to immunoassays, LC-MS/MS based analysis often involves space-consuming and expensive infrastructure [37,38]. Hence, more affordable, accessible, and portable technologies with a rapid turnaround are greatly needed to enhance the feasibility of implementation of TDM in the clinic. SERS-based methodologies may be one such solution.

In order to quantitate drug exposure, a variety of parameters may be interrogated. Peak concentrations of drugs, or Cmax, typically achieved through collection of biosamples 1–2 h after drug administration, are important for some drugs. For chronically administered drugs, the nadir steady-state concentration, or Ctrough, obtained through sample collection prior to drug dosing, is commonly used. The area under the curve, obtained from the integral of the concentration–time curve, may provide a more accurate understanding of drug exposure and may be important for parentally administered drugs with a short elimination half-life, such as chemotherapeutic agents. However, this requires multiple sample collections and Bayesian modelling to populate an AUC curve, and are more of a research tool than a practical method that can be used in the clinic for cancer patients. SERS may allow for drug quantitation with rapid feedback and may enable direct narrow interval monitoring of drug kinetics at the bedside. Furthermore, SERS offers the capacity for multiplexing, thus permitting the detection of many analytes (e.g., parent drug plus active metabolite) simultaneously [39].

The application of SERS in TDM for various biosamples may be complicated by a number of factors. The degree of Raman scattering may vary according to the characteristics of the incident laser, the optical alignment of the equipment, and the acidity of the sample matrix. Thus, these factors must be standardized. Furthermore, areas of increased signal, also known as "hot spots", may change according to qualitative alterations in the SERS substrate, limiting signal reproducibility [40,41]. However, implementing internal standards may help to alleviate the latter issue [42].

Signals from other compounds present in the sample matrix may also impact performance. Oftentimes, these molecules are present with much higher concentrations than the target drug. Human serum contains in excess of 4000 metabolites, many of which have profound affinity for metal surfaces, thus resulting in robust SERS signals [43]. Strategies to obviate the impact of these interfering substances include either incorporating separation technologies, such as thin layer chromatography, or the impregnation of the metal surfaces with specific binding elements, known as "artificial receptors", for the analytes of interest [44,45].

In addition, pretreatment steps, including protein precipitation, filtration, and solid phase extraction, may be employed, but may require experienced operators and prolong the analysis time. However, there has recently been a demonstration of the capacity for automation through the incorporation of centrifugal microfluidics, thus reducing the time and fiscal downsides to the requirement for sample pretreatment. This technology involves applying centrifugal forces on a rotating disc in order to mix or filter small amounts of fluids. Impregnating SERS substrates into such discs may enable multiplexing and the absolute automation of analysis [46].

Despite these challenges, emerging data warrant further exploration of SERS-based approaches for TDM specifically for anticancer therapies. SERS-based TDM has been recently explored for a number of cytotoxic therapies. Panikar and colleagues [47] devised a SERS-based methodology for the quantitation of the widely used chemotherapeutic paclitaxel and the alkylating agent cyclophosphamide. Their approach demonstrated a four-fold improvement in sensitivity for paclitaxel and a one-fold improvement in sensitivity for cyclophosphamide quantitation compared to LC-MS-based approaches. In a separate venture, Panikar and colleagues [48] also evaluated a SERS-based methodology for doxorubicin quantitation in blood serum. Other agents previously quantified through SERS-based methodologies with performance comparable to existing methods include 6-mercaptopurine [49], methotrexate [50], and irinotecan [51]. In order to improve access to TDM for providers and patients, there has been attention to the potential for analysis of microsamples of blood rather than larger biosamples. Interestingly, Zhang and colleagues [52] demonstrated a rapid-turnaround highly reproducible microfluidic SERS-based analysis for 6-thioguanine.

There has also been interrogation of the putative role for SERS-based TDM for targeted therapies. Imatinib is a multikinase inhibitor which has revolutionized outcomes for chronic myeloblastic leukemia and gastrointestinal stromal tumours. It has emerged as a robust candidate for the implementation of TDM in optimizing outcomes [53]. Recently, in small studies, SERS-based approaches for imatinib quantitation in plasma indicated comparable performance to LC-MS based strategies [54–56]. Other tyrosine kinase inhibitors, such as sunitinib and erlotinib, have also been successfully quantitated in SERS-based approaches in early proof-of-concept work [57,58].

A significant practical limitation of the routine use of TDM in the clinic is that it requires a venous blood sample by venepuncture. This intervention requires attendance at a healthcare facility and also discomfort associated with blood collection, which limits routine use [59,60]. A potential strategy is to perform TDM on blood microsamples obtained with finger prick collected as a dried blood spot (DBS) [61]. This approach enhances stability of the analyte in the matrix, does not require centrifugation, and can be stored at room temperature and transported to the laboratory by post [61]. Therefore, with appropriate patient training, implementation of TDM using a DBS may be possible for patients who

face issues with mobility or access to healthcare facilities [62]. Although a number of obstacles in utilizing DBS as an accurate sample matrix have been raised, including the most frequently cited problem of haematocrit variability, a number of early studies have shown DBS to be a valid alternative to venous blood-derived samples for TDM [63]. Some early works have demonstrated the potential to deploy Raman spectroscopy on cellulose paper [64]. To our knowledge, however, SERS-based TDM has never been attempted on the DBS matrix. Further work in this area is merited, as the benefits of combining these two approaches may revolutionize the feasibility of routine TDM in oncology.

Thus, despite this promising early signal for SERS in TDM, further evaluation in larger samples is required. In addition, given the ever-expanding armamentarium of anti-cancer therapies, delineation of the strongest candidates for TDM using SERS is likely to be a challenging but worthwhile enterprise.

5. The Potential Role for SERS in ctDNA Analysis

Cell-free DNA (cfDNA) is a term referring to extracellular molecules of DNA (either double-stranded or mitochondrial DNA) arising from any cell variant found in body fluids. cfDNA has been demonstrably detected in blood as early as the 1940s [65]. The applications of this technology are broad. Currently, cfDNA detection and analysis may have applications in cancer, transplantation medicine, autoimmune disease, cardiology, infectious diseases, and trauma. There is still a lack of clarity regarding the mechanisms of the origin and clearance of cfDNA. A variety of modalities, including apoptosis, ferroptosis, NETosis, phagocytosis, necrosis, and active secretion, have been previously implicated. Similarly, the mechanisms of clearance of cfDNA also remain nebulous, with probable roles for enzymatic breakdown in the circulation, as well as hepatic and renal clearance [66].

The detection of tumour-derived cfDNA, commonly called circulating tumour DNA (ctDNA), has been an area of massive enthusiasm in oncological research. The detection and quantitation of ctDNA may allow for the nuanced intensification and de-intensification of systemic therapy, as well as the exquisite assessment of cancer response to treatment. A number of parameters have been associated with the quantity of emitted detectable ctDNA. Indeed, the volume, metabolism, and proliferative rates have been positively associated with the amount of ctDNA in plasma [67–69].

Despite the potential to detect actionable specific aberrations, given the high frequency of epigenetic changes evident in various cancers, including copy number alterations, chromosomal rearrangements, methylation, fragmentation, and gene expression, there is ongoing research into characterizing these changes as well [70]. These directed approaches for the detection of pre-specified mutations can be undertaken in a highly sensitive and specific manner by utilizing approaches such as RT-PCR, droplet digital PCR (ddPCR), or BEAMing dPCR. Other modalities for ctDNA detection aim to demystify multiple mutations contemporaneously and may vary from identifying tens of mutations to genome-wide interrogation through whole-exome sequencing (WES) or whole-genome sequencing (WGS). These techniques largely rely upon next-generation sequencing (NGS), but mass-spectrometry-based isolation of PCR amplicons is also gaining traction [71]. These more comprehensive strategies of genetic characterization permit assessments of molecular heterogeneity between tumour deposits in individual patients as well as over time, which may be utilized to detect resistant mutations prior to clinical progression [67,72,73].

5.1. Applications for ctDNA Analysis in Oncology

There are emerging data regarding the current role of ctDNA analysis in oncology, spanning the early stage to the metastatic setting for an array of malignancies. These are pictorially represented in Figure 2. In localized cancers, a significant challenge is the limits of detection of radiological studies, and, as such, the identification of residual disease prior to recurrence, with the appropriate application of neoadjuvant or adjuvant systemic therapy. ctDNA may offer a solution. If ctDNA enables the reliable detection of a small number of otherwise undetectable cancer cells following curative-intent treatment, the

concept of "minimal residual disease", or MRD, historically coined in reference to BCR-ABL fusion detection in the monitoring of chronic myeloblastic leukemia, may be applicable in the management of all malignancies [74]. Novel data have suggested a role for such an approach with attendant prognostic and therapeutic implications in the management of a number of solid organ malignancies.

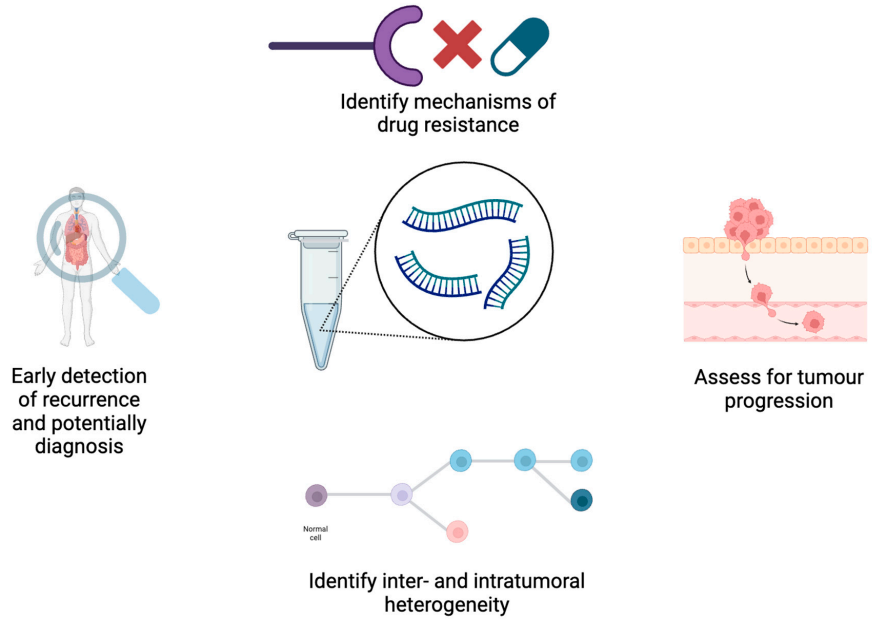

Figure 2. Potential roles for ctDNA analysis in oncology.

For instance, in a subgroup of patients undergoing definitive chemoradiotherapy for locally advanced non-small cell lung cancer, adjuvant immunotherapy offered a benefit in recurrence prevention to patients with detectable ctDNA before chemoradiotherapy, but did not offer the same to patients without detectable tumour DNA [75]. In addition, in a subgroup analysis of patients with muscle-invasive bladder cancer, a condition with a significant chance of relapse post curative-intent surgery, adjuvant immunotherapy demonstrated a benefit only in those with identifiable ctDNA after surgery [76]. Beyond post hoc exploratory analyses, there are also intriguing data in the prospective space. The DYNAMIC trial randomized around 300 patients after surgery for stage II colon cancer to a ctDNA-guided approach to post-operative treatment, or provision of treatment based on conventional prognostic and predictive factors. In the ctDNA-guided cohort, adjuvant chemotherapy or surveillance was based on ctDNA detection on two collections post-operatively. The population of patients with the ctDNA-guided approach received less adjuvant treatment compared to the standard arm, without a negative impact on rates of relapse [77]. Hence, ctDNA detection may be a valuable biomarker in enabling the intensification or de-intensification of neoadjuvant or adjuvant systemic treatment, maximizing the chance of cure for patients with localized cancer, while obviating unnecessary toxicity.

There are also roles for ctDNA in the domain of advanced malignancy. Metastatic malignancy is a dynamic biological process involving the progressive evolution of various subclones with disparate biology. Indeed, the paradigm of intra- and inter-tumoral molecular heterogeneity has been well-described for a number of cancers [78]. Although repeated tissue collection using the biopsy of specific tumours may enable a degree of appreciation for these alterations and sometimes facilitate effective changes in treatment, these strategies are limited by the requirement to obtain fresh tumour samples. Additionally, there is a significant chance that the selected biopsy is not representative of the biology of the entire burden of cancer within an individual. Conversely, liquid biopsy offers the putative benefits of providing a more comprehensive representation of the varied tumour biology at

play, and also can be performed more frequently due to the less invasive nature of blood collection [79–81].

ctDNA may also be highly useful in assessing the response to systemic therapy, particularly in the early phase of treatment. ctDNA has a short circulating half-life and, therefore, provides a real-time assessment of therapeutic response. Conventionally, response assessment is often predicated on repeated radiological assessment. These approaches are limited by allowing for a sufficient timeframe in order to draw meaningful conclusions, as well as complexities around imaging interpretation when multimodal therapies are used (e.g., radiotherapy), or when effective treatments may result in the phenomenon of initial 'flare' of existing lesions, or pseudoprogression (e.g., immunotherapy). Hence, early objective response assessment through repeated liquid biopsy may allow for the early detection of resistance to ineffective systemic approaches, permitting an earlier switch to an alternative therapy [82].

In addition to detecting progression itself, ctDNA analysis may define mechanisms of resistance underlying cancer progression. There are numerous contemporary examples of the efficacy of modifying therapy predicated on specific targetable molecular alterations promoting resistance to current treatment. For instance, the estrogen receptor 1 (ESR1) mutation may be associated with resistance to aromatase inhibitors in breast cancer. The preferential utilization of selective estrogen receptor degraders, such as fulvestrant, in this setting, is associated with improved outcomes [83].

There are also potential roles for the utilization of ctDNA analysis prior to the diagnosis of cancer. There are early data that suggest a role for ctDNA analysis in cancer screening [84]. In patients with suspected malignancy based on radiological studies, obtaining samples of tumour tissue may be anatomically challenging and analysis may be delayed. Testing for ctDNA may offer a more rapid turnaround to safely obtain a diagnosis and potentially enact appropriate therapy more quickly. This is particularly germane in driver-mutation non-small cell lung cancer (NSCLC), where detection of specific molecular aberrations completely alter the treatment approach and outcomes. The ACCELERATE study demonstrated that the time to treatment in this population could be cut down by around half by relying on ctDNA testing for actionable mutations, rather than awaiting somatic mutation analyses on tissue [85].

5.2. Current Obstacles to Clinical Implementation of ctDNA Analysis in Oncology

Despite the numerous potential applications for ctDNA analysis in oncology, there are an array of obstacles. Obviously, the first challenge is to delineate and validate the specific areas whereby ctDNA analysis may be most appropriate for implementation. Technical issues may include fiscal barriers, timely results, and improving sensitivity and specificity for the detection of minimal residual disease status and primary cancer screening. Traditional approaches, including Sanger sequencing and real-time PCR, are relatively insensitive, with the threshold analyte concentration for detection being 15–20% and 1% respectively [86,87]. Given the minuscule concentrations of ctDNA in blood, these limitations are prohibitive. In contrast, digital droplet PCR (ddPCR) and BEAMing (beads, emulsion, amplification, and magnetics)-based approaches permit detection of 0.01% mutant allele frequency of ctDNA. However, these strategies only allow for the detection of specified point mutations. Next-generation sequencing (NGS)-based strategies obviate this issue, enabling the simultaneous detection of multiple cancer mutations in ctDNA isolation, but are costly and time-consuming. Hence, novel sensitive and specific tools amenable to multiplexing that are low cost and are feasible for bedside testing are greatly needed. SERS-based strategies may provide a solution.

5.3. The Role of SERS in ctDNA Detection

It has been previously demonstrated that deploying a SERS-PCR approach is feasible and effective for multiplexed ctDNA detection in a plethora of settings [88]. These strategies enable the detection of specific point mutations with a high degree of sensitivity through

generation of a localized surface plasmon resonance (SPR) indicative of the corresponding molecular fingerprint associated with the mutation of interest [51]. Furthermore, the narrow width of Raman bands facilitates profound specificity in the detection of multiple target molecules [89]. A number of research initiatives have involved the concomitant use of PCR amplification with SERS [90,91], while the other published literature describes approaches involving amplification-free mutation detection [92]. The specific mechanistic aspects around ctDNA biosensing via SERS-based techniques are beyond the scope of the current clinically oriented opinions and have been extensively described in the other literature [93].

SERS-based approaches have been validated in the ctDNA detection for a variety of solid organ malignancies. In colorectal cancer, the presence of mutations in Kirsten rat sarcoma virus (KRAS) BRAF and mutations have significant prognostic and predictive implications [94]. Hence, methods which enable a more timely isolation of these molecular aberrations, potentially from ctDNA, may permit a more rapid implementation of appropriate therapy. This approach was first validated in melanoma, whereby PCR-based amplification was combined with SERS nanoprobes for isolating specific mutations (BRAF V600E, NRAS Q61K and c-kit L756P). This approach was able to detect under 5 ng of ctDNA in serum samples from melanoma patients [95]. Lyu and colleagues demonstrated that SERS nanotags complexed with a short oligonucleotide sequence capable of hybridizing with PCR amplicons corresponding to point mutations in KRAS and BRAF permitted the detection of as few as 0.1% mutant alleles among abundant wild-type ctDNA and compared favourably against ddPCR [96]. The detection of BRAF V600E and IDH1 R132H mutations in ctDNA utilizing a SERS analysis platform in glioblastoma mouse models was shown to be highly sensitive [97]. These studies are summarized in the Table 1 below.

Table 1. Existing data evaluating ctDNA detection using SERS-based methodologies.

Cancer Type and Population	SERS Methodology	Mutations Detected	Detection Limit	Reference
Melanoma, humans	SERS/multiplex PCR	BRAF V600E, NRAS Q61K, c-kit L756P	<5 ng	Wee et al., 2016 [95]
Colorectal cancer, humans	SERS/multiplex PCR	KRAS G12V, G13D, BRAF V600E	0.1%	Lyu et al., 2020 [96]
Glioblastoma, mouse model	SERS/catalytic hairpin assembly	BRAF V600E, IDH R132H	~6×10^{-18} mol (6 aM)	Wang et al., 2023 [97]

6. Conclusions and Perspective

Currently, the strategies of therapeutic drug monitoring and ctDNA detection hold great promise in transforming cancer care. However, there is a significant clarification required in terms of the best settings in which to incorporate these approaches. The needs of the research required to clarify these pressing questions are likely to be prodigious, and it is important to identify cost-effective, practical, and rapid methods for TDM and ctDNA detection. Thus, given the time- and cost-efficient nature of SERS-based approaches in relation to existing "gold-standard" techniques, these methodologies should be explored. Indeed, although such ambitious research into this esoteric and highly complex area is likely to be challenging, the breadth of the potential applications within oncology, as well as medicine as a whole, necessitate further interrogation into this potentially transformative tool.

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