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Predicting Radiotherapy Responses and Treatment Outcomes Through Analysis of Circulating Tumor DNA

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Tumors continually shed DNA into the blood where it can be detected as circulating tumor DNA (ctDNA). Although this phenomenon has been recognized for decades, techniques that are sensitive and specific enough to robustly detect ctDNA have only become available recently. Quantification of ctDNA represents a new approach for cancer detection and disease burden quantification that has the potential to revolutionize response assessment and personalized treatment in radiation oncology. Analysis of ctDNA has many potential applications, including detection of minimal residual disease following radiotherapy, non-invasive tumor genotyping, and early detection of tumor recurrence. Ultimately, ctDNA-based assays could lead to personalization of therapy based on identification of somatic alterations present in tumors and changes in ctDNA concentrations before and after treatment. In this review, we discuss methods of ctDNA detection and clinical applications of ctDNA-based biomarkers in radiation oncology, with a focus on recently developed techniques that use next-generation sequencing for ctDNA quantification.
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Introduction

The field of radiation oncology is currently going through a revolution, as new technologies allow treatment of patients with more precision than ever before. Although much progress has been made in improving delivery of radiation therapy (RT), response assessment after treatment continues to rely primarily on imaging for most types of cancers. Although, imaging-based response assessment has significant clinical value, this approach cannot detect minimal residual disease (MRD), has a lag between treatment and when tumor responses can be detected, is expensive, and often exposes patients to additional ionizing radiation. For these reasons, there continues to be an unmet need for developing blood-based biomarkers for cancers that are treated with RT. Although such biomarkers exist for a few cancer types (eg, prostate-specific antigen for prostate cancer), they do not for most cancers. The ideal biomarker would be readily generalizable, such that it could be applied to all cancer types.

Although most work on cancer biomarkers has focused on detection of proteins, recently, circulating tumor DNA

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(ctDNA) has emerged as a plasma-based biomarker with the potential to redefine prediction and monitor the response to anticancer therapies. The cell-free component of whole blood has long been recognized to contain fragmented DNA molecules[1] that are collectively referred to as cell-free DNA (cfDNA). These molecules are derived from dying cells, and in healthy individuals, they mainly originate from hematopoietic cells,[2-4] although diverse tissues contribute to the total cfDNA pool. Concentrations of cfDNA in plasma of healthy individuals range from 1-10 ng/ml. Patients with advanced malignancies can display elevated levels of cfDNA, which led several early studies to suggest that measurement of total cfDNA concentration may be a useful biomarker for detection and monitoring of cancers.[5-9] However, further work revealed that a variety of nonmalignant physiological states and stresses can lead to dramatic elevations of cfDNA concentrations, including exercise, trauma, infection, and inflammation.[10-12] Furthermore, as detailed later, the fraction of cfDNA that is derived from tumors in patients with cancer is often extremely low. Thus, the measurement of total cfDNA concentration does not have sufficient specificity or sensitivity to serve as a reliable cancer biomarker.

Circulating Tumor DNA

Malignant tumors continually shed DNA into the circulation.[13-18] The mechanisms of release of nucleic acid into the blood are not well understood but are thought to result from necrosis, apoptosis, and release of DNA by phagocytes that have engulfed tumor cells (Fig. 1).[17,19-21] Importantly, most of the cfDNA found in plasma of patients with cancer usually originates from nonmalignant cells. This is often the case even for patients with metastatic disease. For example, in patients with metastatic non–small cell lung cancer (NSCLC), pretreatment ctDNA percentages can be less than 1% at diagnosis, and decline if active treatment is initiated.[16,18,22] Similarly, when Dawson et al[14] quantified ctDNA in patients with metastatic breast cancer, the median mutant allele fraction was ∼4%. This problem is magnified in patients with earlier stages of cancer and lower disease burdens, as well as in settings where no gross disease remains after therapy and microscopic residual disease must be detected. Thus, only highly sensitive detection techniques are likely to be of clinical use.[13,23] Here we describe, compare and contrast methods to detect ctDNA (Table).

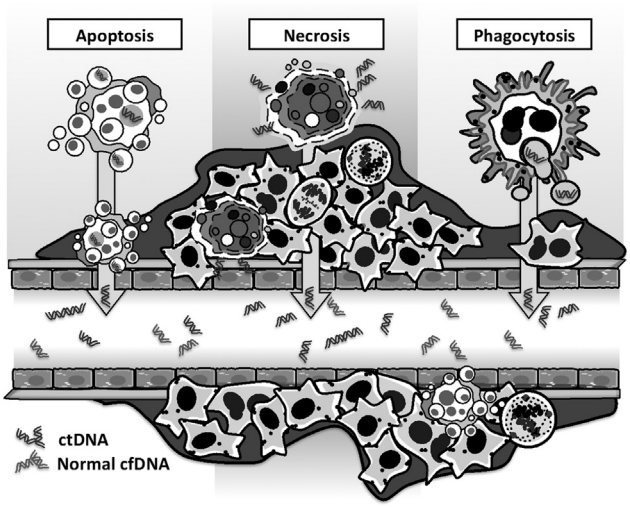

Figure 1 Sources of circulating tumor DNA. Tumor DNA is released into blood predominantly by tumor cell death mechanisms including necrosis and apoptosis. Phagocytes that have engulfed tumor cells may also actively release tumor DNA into the extracellular space.

Allele-Specific PCR–Based Methods for ctDNA Detection

Initial efforts to quantitate ctDNA focused on detection using polymerase chain reaction (PCR)–based methods such as quantitative PCR or PCR approaches that can preferentially amplify low levels of mutant alleles in the presence of large amounts of wild-type DNA. Examples of the latter include coamplification at lower denaturation temperature-PCR (COLD-PCR), peptide nucleic acids, locked nucleic acids, and amplification-refractory mutation systems. These approaches have analytic sensitivities in the range of 0.1%-1%.[24] Studies using such approaches documented the presence of somatic mutations in genes such as KRAS or APC in the plasma of patients with a variety of cancer types.[25-27] Similarly, it was shown that PCR-based methods could detect microsatellite alterations in cfDNA.[28] Advantages of PCR-based approaches include low cost and relative ease of use. However, the key limitation of these methods is that they are usually not sufficiently sensitive to detect ctDNA in patients with early-stage disease or patients with advanced disease who are responding to therapy. Consequently, a number of early studies concluded that ctDNA was unlikely to be a clinically useful biomarker, which for a period decreased interest in the field.[21,29,30]

Digital PCR-Based ctDNA Detection Methods

In the last several years, there has been a renewed interest in developing ctDNA as a clinical biomarker because of the addition of 2 major classes of techniques for ctDNA detection. The first of these classes was digital PCR (dPCR), which allows more precise quantitation of minor mutant allele fractions than the aforementioned PCR-based techniques. The 2 most prominent examples of dPCR-based methods are beads, emulsion, amplification, and magnetics (BEAMing[27]) and droplet dPCR.[27,31] Both the methods involve separation of template molecules into thousands of tiny droplets that each represents isolated reaction chambers, which contain at most 1 template molecule. Paired with allele-specific PCR assays, this allows extremely precise enumeration of the number of total template molecules that carry a mutation of interest and can achieve detection limits as low as 0.01%. Using these methods,

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Table Comparison of Methods for Detecting Circulating Tumor DNA

	Allele-Specific PCR	Digital PCR	NGS Amplicon Based	WGS	WES	CAPP-Seq
Method	Preferentially amplifies rare mutant DNA molecules	Counts mutant molecules via partitioning of DNA molecules	Deep sequencing of PCR amplicons	Deep sequencing of entire genome	Deep sequencing of exome	Targeted hybrid capture
Detection limit (as % ctDNA)	~0.1%-1%	~0.01%	~0.01%-2.0%	~1%	~5%	~0.01%
Advantages	Ease of use	High sensitivity	High sensitivity (some methods)	Entire genome is interrogated	Entire exome is interrogated	High sensitivity Detects SNVs, indels, rearrangement, and SCNAs
	Lowest cost		Less expensive than other NGS methods	Broadly applicable without personalization	Broadly applicable without personalization	Broadly applicable without personalization
Limitations	Lower sensitivity	Can only test small number of genomic positions in a sample	Less comprehensive than other NGS methods	Expensive	Expensive	Less comprehensive than WGS or WES
	Can only test small number of genomic positions in a sample		Inability to detect SCNAs	Low sensitivity	Low sensitivity	
			Inability to detect rearrangements without assay personalization	Mostly limited to SCNA detection		

Abbreviations: indels, insertions or deletions; NGS, next-generation sequencing; PCR, polymerase chain reaction; SCNA, somatic copy number alteration; SNV, single nucleotide variation; WES, whole-exome sequencing; WGS, whole-genome sequencing.

ctDNA has been detected in a wide range of patients with cancer.[22,32-34]

The dPCR techniques are currently the gold-standard methods for detection of specific mutations within ctDNA, given their high sensitivity, specificity, and relatively low cost. However, an important limitation of these approaches is that only a single or a small number of mutations can be interrogated at a time. Owing to the low concentration of ctDNA found in many patients, often an entire 10-20 mL of blood draw needs to be exhausted for evaluation of only a handful of mutations. Additionally, to use dPCR to monitor treatment responses, at least one mutation must first be identified via analysis of tumor tissue, thus usually requiring an initial invasive procedure.

Next-Generation Sequencing–Based ctDNA Detection Assays

Owing to the practical limitation of the number of genomic positions that can be interrogated by dPCR, a number of groups recently focused efforts on developing next-generation sequencing (NGS)–based approaches for ctDNA detection. NGS allows for massively parallel sequencing of hundreds of millions of DNA fragments from a single sample and thus opens the possibility of genotyping many potential mutations at a time. The NGS-based approaches can be categorized based on the number of genomic regions they interrogate as either being "focused" or "broad."

The focused NGS-based approaches have mainly used PCR amplicon-based strategies. Specifically, PCR is used to amplify a handful of regions of the genome, and the resulting amplified products are subjected to NGS. For example, Forshew et al[15] developed Tagged-Amplicon Sequencing (TAm-Seq) and used it to detect mutations in plasma samples from patients with advanced ovarian cancer, showing that this technique can achieve a detection limit of ~2%. In parallel, Narayan et al developed an amplicon-based method that incorporates an error-suppression approach. Focusing on hotspots in EGFR, BRAF, and KRAS, they showed that they could detect mutations in these genes in the plasma of some patients with lung cancer.[35] Finally, Kinde et al[36] developed an amplicon-based approach called the Safe-Sequencing System (Safe-SeqS), which uses a barcoding approach to suppress errors and thus increase sensitivity while maintaining high specificity. This method was recently successfully applied to detect ctDNA in a large variety of tumor types.[13] Key advantages of the amplicon-based approaches are that they are relatively inexpensive and sensitive, especially if error-suppression strategies are used. Limitations of these approaches include interrogation of relatively few genomic loci owing to difficulties related to multiplexing of PCR assays, the inability to detect somatic copy

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number alterations, and the inability to detect breakpoints or rearrangements without patient-specific optimization of amplicons.

The "broad" NGS-based ctDNA detection methods include whole-exome sequencing (WES) and whole-genome sequencing (WGS). WES involves sequencing of all exons within the genome using approaches routinely used for tumor genotyping. Murtaza et al published the first application of this approach and conducted serial sampling of plasma from 6 patients with metastatic breast cancer, ovarian cancer, and NSCLC who were undergoing systemic therapy. Multiple time points were analyzed, and samples were preselected to contain high percentages of ctDNA (between 5% and 55%). Results revealed that many of the mutations detected in the plasma matched those in biopsies of metastatic deposits. Additionally, they found that new mutations arose as patients developed resistance to therapy.[37] The major limitations of WES for ctDNA analysis are its high cost and relatively low sensitivity, making it applicable primarily for research purposes in patients with high burden of metastatic disease.

Finally, several groups have performed WGS directly on cfDNA from patients with cancer. Leary et al used WGS and identified copy number alterations and chromosomal rearrangements in 9 of 10 patients with colorectal and breast cancers, with ctDNA fractions ranging between 1.4% and 48% of total cfDNA. In a modeling exercise, maximum sensitivity of this approach was predicted to be $>90\%$ at a specificity $>99\%$ when ctDNA levels were $\geq 0.75\%$.[38] Other groups have similarly performed WGS on plasma samples from patients with metastatic prostate cancer or hepatocellular carcinoma, indicating that this approach is widely applicable.[39,40] Strengths of the WGS approach include that it does not restrict the sequencing space. Limitations include high cost owing to the amount of sequencing required, and dependence primarily on copy number variation analysis, with inadequate sensitivity to detect specific single nucleotide variations, insertions, deletions, or rearrangements.[38,39]

Development of Cancer Personalized Profiling by Deep Sequencing

To take advantage of the key strengths of both the "focused" and "broad" ctDNA detection approaches described earlier, we recently developed a novel method called *Cancer Personalized Profiling by Deep Sequencing* (CAPP-Seq). This approach allows ultraspecific and ultrasensitive quantification of tumor-derived ctDNA (detection limit $\sim 0.01\%$), is economical, and is generalizable to nearly all tumor types (Fig. 2).[16] The method allows detection of specific single nucleotide variations, insertions or deletions, somatic copy number alterations, and gene rearrangements in a single assay. CAPP-Seq uses a multiphase bioinformatics approach using existing genomic data to design a variably sized "selector" consisting of tagged nucleic acids that allow enrichment for recurrently mutated regions in the cancer of interest via hybrid capture.[16,23] The same selector is applied to all patients with a given tumor type, allowing identification of multiple mutations per tumor without the need for personalization of assays. To use CAPP-Seq for detecting or monitoring ctDNA, a sequencing library is prepared from cfDNA, the selector is applied to enrich for genomic regions of interest, and the resulting enriched library is subjected to NGS.

We initially developed CAPP-Seq for NSCLC and showed that in our initial cohort, it detected ctDNA in all the patients with stage II-IV disease and in 50% of the patients with stage I disease, with 96% specificity.[16] Strengths of CAPP-Seq include that it can simultaneously cover thousands of distinct genomic regions, can detect all major classes of somatic alterations, does not require prior knowledge of a tumor's mutations owing to its breadth of coverage, does not require patient-specific optimization, is economical, and has very high analytic sensitivity. Limitations include that it is somewhat more time intensive than amplicon-based approaches are and that, similar to WES or the focused NGS-based methods, it can only detect alterations in genomic regions that are covered.

Applications of ctDNA Analysis in Radiation Oncology

The development of novel methods for ctDNA detection offers the opportunity to investigate the potential clinical use of incorporating ctDNA analysis into treatment strategies for patients receiving RT. A major advantage of ctDNA when compared with other cancer biomarkers is that it is easily generalizable. For example, protein-based biomarkers depend on the identification of specific proteins that are overexpressed by a cancer of interest and subsequent optimization of detection strategies that are usually antibody based. For this reason, a protein-based biomarker for one cancer type usually does not readily extend to other cancer types. On the contrary,

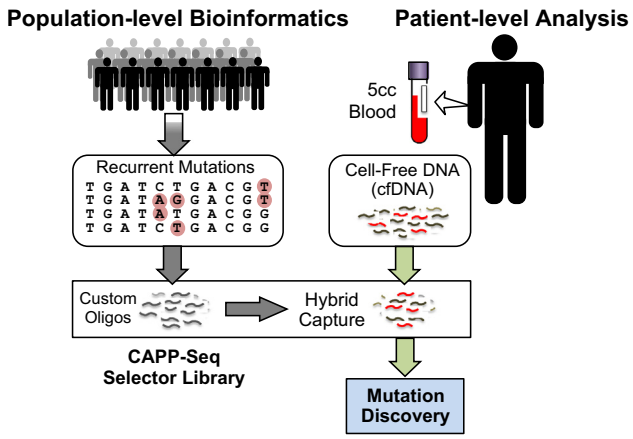

Figure 2 Design and implementation of CAPP-Seq. Bioinformatic analysis of cancer WES or WGS data is used to select recurrently mutated genomic regions for inclusion in a CAPP-Seq selector consisting of biotinylated oligonucleotides. The same CAPP-Seq selector can then be applied in nearly all patients with a given cancer type. The selector is used for hybrid capture followed by next-generation sequencing of circulating tumor DNA. (Color version of figure is available online.)

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as all cancers contain somatic alterations, analysis of ctDNA could potentially be applied to all tumor types. It is possible that successful integration of ctDNA analysis into treatment paradigms for one cancer type could allow for rapid extension of the approach to many other cancer types.

Treatment Response Assessment

A potential application of ctDNA detection in radiation oncology is for treatment response assessment. Currently, imaging represents the mainstay of response assessment after RT. However, surveillance imaging is not without risks; the tests typically performed, computed tomography (CT) and positron emission tomography (PET), expose patients to additional ionizing radiation which, although significantly lower than therapeutic RT doses, often expose a larger fraction of the body, and for patients with frequent surveillance or long follow-up, can summate to a clinically meaningful risk of carcinogenesis.[41-43] Indeed, for an increasing number of diseases, the risks of surveillance imaging are felt to potentially outweigh the benefits.[44] Additionally, post-RT imaging studies are often difficult to interpret because of normal tissue changes such as scarring induced by treatment. This may complicate the interpretation of posttreatment scans and lead to significant subjectivity in determining treatment response or disease status.

As opposed to imaging studies, analysis of ctDNA readily produces quantitative results that can be tracked over time. For example, studies in patients with advanced melanoma, ovarian, breast, lung, and colon cancers have shown precise tumor burden quantification with ctDNA, with elevations in ctDNA concentration strongly correlating with disease progression and ctDNA decline correlating with successful treatment.[14-16,32,45,46] Thus, ctDNA analysis appears to offer an attractive adjunct or potentially an alternative to standard follow-up imaging in patients with advanced disease.

Additionally, early results suggest that ctDNA analysis likely is more sensitive than conventional imaging studies are for detection of disease progression. For example, Dawson et al[14] showed that in patients with metastatic breast cancer with increasing ctDNA levels during surveillance, progression was identified on an average of 5 months before imaging. Similarly, several recent studies focusing on EGFR mutant advanced NSCLC have shown that in many cases, progression can be detected via elevations in ctDNA levels months before changes on imaging studies.[18,47] Thus, ctDNA quantification offers several potential benefits over standard imaging for cancer surveillance.

Few studies to date have explored the application of ctDNA analysis for surveillance after RT. We recently provided several examples of using CAPP-Seq to distinguish between normal tissue changes and residual disease in patients with NSCLC treated with fractionated RT or stereotactic ablative RT.[16] These results suggest that analysis of ctDNA may have clinical utility in aiding the interpretation of post-RT imaging studies. Alternatively, if it is shown that ctDNA analysis has sufficient sensitivity, one could envision using it as the primary surveillance assay and performing imaging only when there is evidence of increasing ctDNA levels or when patients develop new symptoms. The latter approach is analogous to the use of prostate-specific antigen to follow up patients treated with definitive RT for prostate cancer and thus has clinical precedent.

Detection of MRD

The concept of MRD is well established in hematologic malignancies and refers to microscopic deposits of malignant cells present after therapy in patients who do not have any symptoms or imaging findings confirming their presence. MRD is responsible for tumor relapse and can only be detected with modern and extremely sensitive molecular methods.[48] Although similar methods currently do not exist for most solid tumors, quantification of ctDNA could potentially allow for the detection of MRD after treatment with RT, because it is possible that patients with residual ctDNA after definitive treatment would be enriched for patients who will ultimately develop recurrence. An example that suggests this may be feasible comes from a recent study of ctDNA in patients with stage II colorectal cancer treated with surgery. Tie et al[49] reported that in their cohort, 5 of 6 patients with detectable postoperative ctDNA developed recurrence, whereas only 5 of 72 patients with no residual ctDNA had recurrence. These data suggest that residual ctDNA after curative intent treatment may provide a means to identify the subset of patients who are at the highest risk for recurrence.

The most significant body of literature indicating that a similar approach may be fruitful in patients treated with RT comes from the analysis of circulating Epstein-Barr virus (EBV) DNA in patients with nasopharyngeal carcinoma treated with chemoRT. Development of nasopharyngeal carcinoma is strongly associated with EBV, and circulating EBV DNA levels are detectable in many patients before RT. Successful treatment results in loss of circulating EBV DNA, whereas detection of EBV DNA in the circulation after therapy portends significantly worse overall and relapse-free survival.[50-53] Other studies have similarly quantitated circulating human papillomavirus DNA in patients with head and neck cancer.[54,55] Analysis of ctDNA from other cancers is analogous to the analysis of circulating EBV and HPV DNA from head and neck cancers, except that the analyte comes from the cancer genome instead of a cancer-associated virus. For example, our initial description of CAPP-Seq included an example of a patient with stage III NSCLC who was definitively treated with chemoRT.[16] Posttreatment imaging revealed an apparent complete response, but ctDNA concentrations did not change, suggesting progression of micrometastatic disease that compensated for the volume of tumor eliminated by RT. This patient developed metastases in multiple organs several months later, clinically confirming the ctDNA result. Thus, ctDNA analysis could potentially facilitate the identification of patients with MRD after RT. In this scenario, this would facilitate the development of clinical trials that test escalating therapy for patients with residual disease (eg, with adjuvant chemotherapy, targeted therapy, or immunotherapy) and de-escalation of therapy for patients without residual disease (eg, by eliminating adjuvant chemotherapy in clinical situations where it is currently given to all patients).

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Analysis of ctDNA Kinetics for RT Response Prediction

Acute changes in ctDNA concentrations during RT or immediately following treatment might also have a prognostic or predictive value. As ctDNA release is a result of cell death, cell killing by RT could potentially be monitored via changes in ctDNA concentration. Given that the half-life of ctDNA in the circulation is approximately 0.5-2 hours,[32] only tumor cells that died several hours before sample collection contribute to ctDNA levels, and early changes in ctDNA levels after initiation of RT might be useful for predicting the ultimate response to treatment. Indeed, Lo et al[56] showed that for nasopharyngeal carcinoma treated definitively with RT, plasma EBV DNA levels elevated during the first week of treatment (suggesting an increase in rate of cell death) and subsequently declined (suggesting decreasing tumor burden). In some patients, the initial elevation occurred immediately following treatment initiation, whereas in others, the elevation occurred later in the week. It is therefore possible that ctDNA level changes early during a course of RT may predict treatment outcome and that early analysis of ctDNA kinetics during treatment could allow clinicians to modify RT and add adjuvant systemic therapy, thus facilitating delivery of truly personalized RT regimens.

Noninvasive Tumor Genotyping

The application of ctDNA analysis that is likely to achieve routine clinical use first is tumor genotyping via the plasma, an approach referred to as noninvasive tumor genotyping. This application is particularly attractive in situations where tumor biopsy is medically risky or all biopsy tissue was exhausted in establishing a diagnosis, which are 2 scenarios frequently encountered in patients who are candidates for RT. Additionally, as blood draws are easily repeated, noninvasive tumor genotyping via ctDNA analysis allows monitoring of genomic evolution of tumors over the course of therapy.

Numerous studies have provided evidence that this application is feasible. For example, Murtaza et al[37] performed serial ctDNA analysis for patients with advanced cancers using WES and showed emergence of mutant alleles at the time of treatment failure, including a truncating RB1 mutation in a patient who developed acquired resistance to cisplatin. Analysis of ctDNA has also been used to identify specific somatic resistance mutations, primarily in EGFR and KRAS, following anti-EGFR therapy in patients with lung cancer and colorectal cancer.[13,22,33,34] For example, Diaz et al[33] showed that KRAS mutations arise in patients with previous wild-type colorectal cancer 5-6 months following treatment initiation with anti-EGFR monoclonal antibodies. Similarly, Misale et al[34] showed that in such patients, resistance mutations could be detected in the plasma as early as 10 months before radiographic disease progression. Thus, ctDNA analysis provides a noninvasive approach for monitoring evolution of the tumor genome and predicting resistance to systemic therapy. Detection of resistance mutations before clinical progression provides an opportunity for exploring if early intervention, for example, by adding another agent that targets the resistance mutations or using an agent with a different resistance profile, could improve outcomes.

Noninvasive genotyping could also be useful for identifying currently unexplored resistance mechanisms. This is particularly true for RT, where resistance has been linked to mutations in prosurvival, tumor suppressor, reactive oxygen species, cell cycle checkpoint, and telomerase pathways.[57-59] However, there is a paucity of literature comparing mutation profiles in tumors before RT and after recurrence, in large part owing to the difficulty of obtaining tumor tissue at both time points. Thus, use of ctDNA analysis in patients whose tumors recur after definitive RT could enable the identification of novel resistance mechanisms and ultimately lead to strategies for improving RT outcomes.

Conclusions and Future Directions

Analysis of ctDNA is a noninvasive approach to detect, genotype, and quantify tumor burden that has the potential to be incorporated into the practice of radiation oncology. Clinical applications include prediction of radiation treatment response, posttreatment disease surveillance, and resistance mutation detection. We envision that in the future, ctDNA assays may be used to personalize RT treatment based on ctDNA levels, kinetics, and tumor genetics. In the future, ctDNA analysis may also complement or in some cases supplement traditional imaging–based and pathology-based staging systems for risk stratification and selection of therapy. Given the large number of potential applications, it is anticipated that there will be a significant number of studies published on ctDNA in the upcoming years exploring the use of ctDNA in a variety of different clinical contexts. Identification of the optimal technical approach for ctDNA assessment in different clinical situations is critical. Finally, as with any proposed cancer biomarker, well-designed and prospective clinical trials need to be performed to fully explore the clinical applications of ctDNA in patients treated with RT.

References

1. Mandel P, Metais P: Les acides nucleiques du plasma sanguin chez lhomme. Cr Soc Biol 142:241-243, 1948
2. Anker P, Stroun M, Maurice PA: Spontaneous release of DNA by human blood lymphocytes as shown in an in vitro system. Cancer Res 35:2375-2382, 1975
3. Stroun M, Lyautey J, Lederrey C, et al: About the possible origin and mechanism of circulating DNA apoptosis and active DNA release. Clin Chim Acta 313:139-142, 2001
4. Rogers JC, Valeri CR, Boldt D, et al: Excretion of DNA by phytohemagglutinin-stimulated lymphocytes. J Clin Invest 51:A80, 1972
5. Wimberger P, Roth C, Pantel K, et al: Impact of platinum-based chemotherapy on circulating nucleic acid levels, protease activities in blood and disseminated tumor cells in bone marrow of ovarian cancer patients. Int J Cancer 128:2572-2580, 2011
6. Boddy JL, Gal S, Malone PR, et al: Prospective study of quantitation of plasma DNA levels in the diagnosis of malignant versus benign prostate disease. Clin Cancer Res 11:1394-1399, 2005
7. Jung K, Stephan C, Lewandowski M, et al: Increased cell-free DNA in plasma of patients with metastatic spread in prostate cancer. Cancer Lett 205:173-180, 2004

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8. Kamat AA, Baldwin M, Urbauer D, et al: Plasma cell-free DNA in ovarian cancer: An independent prognostic biomarker. Cancer 116:1918-1925, 2010
9. Schwarzenbach H, Stoehlmacher J, Pantel K, et al: Detection and monitoring of cell-free DNA in blood of patients with colorectal cancer. Ann N Y Acad Sci 1137:190-196, 2008
10. Atamaniuk J, Vidotto C, Tschan H, et al: Increased concentrations of cell-free plasma DNA after exhaustive exercise. Clin Chem 50:1668-1670, 2004
11. Laktionov PP, Tamkovich SN, Rykova EY, et al: Extracellular circulating nucleic acids in human plasma in health and disease. Nucleosides Nucleotides Nucleic Acids 23:879-883, 2004
12. Lo YM, Rainer TH, Chan LY, et al: Plasma DNA as a prognostic marker in trauma patients. Clin Chem 46:319-323, 2000
13. Bettegowda C, Sausen M, Leary RJ, et al: Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med 6:224ra24, 2014
14. Dawson SJ, Tsui DW, Murtaza M, et al: Analysis of circulating tumor DNA to monitor metastatic breast cancer. N Engl J Med 368: 1199-1209, 2013
15. Forshew T, Murtaza M, Parkinson C, et al: Noninvasive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA. Sci Transl Med 4:136ra68, 2012
16. Newman AM, Bratman SV, To J, et al: An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. Nat Med 20:548-554, 2014
17. Schwarzenbach H, Hoon DS, Pantel K: Cell-free nucleic acids as biomarkers in cancer patients. Nat Rev Cancer 11:426-437, 2011
18. Oxnard GR, Paweletz CP, Kuang Y, et al: Noninvasive detection of response and resistance in EGFR-mutant lung cancer using quantitative next-generation genotyping of cell-free plasma DNA. Clin Cancer Res 20:1698-1705, 2014
19. Jahr S, Hentze H, Englisch S, et al: DNA fragments in the blood plasma of cancer patients: Quantitations and evidence for their origin from apoptotic and necrotic cells. Cancer Res 61:1659-1665, 2001
20. Choi JJ, Reich 3rd CF, Pisetsky DS: The role of macrophages in the in vitro generation of extracellular DNA from apoptotic and necrotic cells. Immunology 115:55-62, 2005
21. Diaz Jr. LA, Bardelli A: Liquid biopsies: Genotyping circulating tumor DNA. J Clin Oncol 32:579-586, 2014
22. Taniguchi K, Uchida J, Nishino K, et al: Quantitative detection of EGFR mutations in circulating tumor DNA derived from lung adenocarcinomas. Clin Cancer Res 17:7808-7815, 2011
23. Newman AM, Bratman SV, Stehr H, et al: FACTERA: A practical method for the discovery of genomic rearrangements at breakpoint resolution. Bioinformatics 30:3390-3393, 2014
24. Milbury CA, Li J, Makrigiorgos GM: PCR-based methods for the enrichment of minority alleles and mutations. Clin Chem 55:632-640, 2009
25. Sorenson GD, Pribish DM, Valone FH, et al: Soluble normal and mutated DNA sequences from single-copy genes in human blood. Cancer Epidemiol Biomarkers Prev 3:67-71, 1994
26. Vasioukhin V, Anker P, Maurice P, et al: Point mutations of the N-ras gene in the blood plasma DNA of patients with myelodysplastic syndrome or acute myelogenous leukaemia. Br J Haematol 86:774-779, 1994
27. Diehl F, Li M, Dressman D, et al: Detection and quantification of mutations in the plasma of patients with colorectal tumors. Proc Natl Acad Sci U S A 102:16368-16373, 2005
28. Nawroz H, Koch W, Anker P, et al: Microsatellite alterations in serum DNA of head and neck cancer patients. Nat Med 2:1035-1037, 1996
29. Daniotti M, Vallacchi V, Rivoltini L, et al: Detection of mutated BRAFV600E variant in circulating DNA of stage III-IV melanoma patients. Int J Cancer 120:2439-2444, 2007
30. Morgan SR, Whiteley J, Donald E, et al: Comparison of KRAS Mutation assessment in tumor DNA and Circulating free DNA in plasma and serum samples. Clin Med Insights Pathol 5:15-22, 2012
31. Hindson BJ, Ness KD, Masquelier DA, et al: High-throughput droplet digital PCR system for absolute quantitation of DNA copy number. Anal Chem 83:8604-8610, 2011
32. Diehl F, Schmidt K, Choti MA, et al: Circulating mutant DNA to assess tumor dynamics. Nat Med 14:985-990, 2008
33. Diaz Jr. LA, Williams RT, Wu J, et al: The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. Nature 486:537-540, 2012
34. Misale S, Yaeger R, Hobor S, et al: Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. Nature 486:532-536, 2012
35. Narayan A, Carriero NJ, Gettinger SN, et al: Ultrasensitive measurement of hotspot mutations in tumor DNA in blood using error-suppressed multiplexed deep sequencing. Cancer Res 72:3492-3498, 2012
36. Kinde I, Wu J, Papadopoulos N, et al: Detection and quantification of rare mutations with massively parallel sequencing. Proc Natl Acad Sci U S A 108:9530-9535, 2011
37. Murtaza M, Dawson SJ, Tsui DW, et al: Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. Nature 497:108-112, 2013
38. Leary RJ, Sausen M, Kinde I, et al: Detection of chromosomal alterations in the circulation of cancer patients with whole-genome sequencing. Sci Transl Med 4:162ra54, 2012
39. Heitzer E, Ulz P, Belic J, et al: Tumor-associated copy number changes in the circulation of patients with prostate cancer identified through whole-genome sequencing. Genome Med 5:30, 2013
40. Chan KC, Jiang P, Zheng YW, et al: Cancer genome scanning in plasma: Detection of tumor-associated copy number aberrations, single-nucleotide variants, and tumoral heterogeneity by massively parallel sequencing. Clin Chem 59:211-224, 2013
41. Tarin TV, Sonn G, Shinghal R: Estimating the risk of cancer associated with imaging related radiation during surveillance for stage I testicular cancer using computerized tomography. J Urol 181:627-632, 2009. [discussion 32-3]
42. Frush DP, Donnelly LF, Rosen NS: Computed tomography and radiation risks: What pediatric health care providers should know. Pediatrics 112:951-957, 2003
43. Fazel R, Krumholz HM, Wang Y, et al: Exposure to low-dose ionizing radiation from medical imaging procedures. N Engl J Med 361:849-857, 2009
44. Pingali SR, Jewell SW, Havlat L, et al: Limited utility of routine surveillance imaging for classical Hodgkin lymphoma patients in first complete remission. Cancer 120:2122-2129, 2014
45. Shinozaki M, O'Day SJ, Kitago M, et al: Utility of circulating B-RAF DNA mutation in serum for monitoring melanoma patients receiving biochemotherapy. Clin Cancer Res 13:2068-2074, 2007
46. Bidard FC, Madic J, Mariani P, et al: Detection rate and prognostic value of circulating tumor cells and circulating tumor DNA in metastatic uveal melanoma. Int J cancer 134:1207-1213, 2014
47. Sorensen BS, Wu L, Wei W, et al: Monitoring of epidermal growth factor receptor tyrosine kinase inhibitor-sensitizing and resistance mutations in the plasma DNA of patients with advanced non-small cell lung cancer during treatment with erlotinib. Cancer 120:3896-3901, 2014
48. Schrappe M. Detection and management of minimal residual disease in acute lymphoblastic leukemia. Hematology Am Soc Hematol Educ Program 2014;2014:244-249.
49. Tie JKI, Wang Y, et al: Circulating tumor DNA (ctDNA) as a marker of recurrence risk in stage II colon cancer (CC). J Clin Oncol 32(suppl), 2014. Abstract 11015
50. Lin JC, Wang WY, Chen KY, et al: Quantification of plasma Epstein-Barr virus DNA in patients with advanced nasopharyngeal carcinoma. N Engl J Med 350:2461-2470, 2004
51. Lo YM, Chan LY, Lo KW, et al: Quantitative analysis of cell-free Epstein-Barr virus DNA in plasma of patients with nasopharyngeal carcinoma. Cancer Res 59:1188-1191, 1999
52. Wang WY, Twu CW, Chen HH, et al: Long-term survival analysis of nasopharyngeal carcinoma by plasma Epstein-Barr virus DNA levels. Cancer 119:963-970, 2013

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53. Twu CW, Wang WY, Liang WM, et al: Comparison of the prognostic impact of serum anti-EBV antibody and plasma EBV DNA assays in nasopharyngeal carcinoma. Int J Radiat Oncol Biol Phys 67:130-137, 2007
54. Cao H, Banh A, Kwok S, et al: Quantitation of human papillomavirus DNA in plasma of oropharyngeal carcinoma patients. Int J Radiat Oncol Biol Phys 82:e351-e358, 2012
55. Ahn SM, Chan JY, Zhang Z, et al: Saliva and plasma quantitative polymerase chain reaction-based detection and surveillance of human papillomavirus–related head and neck cancer. JAMA Otolaryngol Head Neck Surg 140:846-854, 2014
56. Lo YM, Leung SF, Chan LY, et al: Kinetics of plasma Epstein-Barr virus DNA during radiation therapy for nasopharyngeal carcinoma. Cancer Res 60:2351-2355, 2000
57. Bao S, Wu Q, McLendon RE, et al: Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 444:756-760, 2006
58. Diehn M, Cho RW, Lobo NA, et al: Association of reactive oxygen species levels and radioresistance in cancer stem cells. Nature 458:780-783, 2009
59. Morrison R, Schleicher SM, Sun Y, et al: Targeting the mechanisms of resistance to chemotherapy and radiotherapy with the cancer stem cell hypothesis. J Oncol 2011:941876, 2011

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