CPQ Cancer (2019) 1:5
Review Article

CIENT PERIODIQUE

The Role of Circulating Tumor DNA in Cancer Management

Ihab Eldessouki[1], Mohamed Magdy[2], Mohamed Kamel[3*], Mohamed Rahouma[3], Adam Sholi, N.[4], Tatyana Zaytseva[5], John Morris, C.[1] & Nagla Abdel Karim[1]

[1]*Hematology-Oncology Department, University of Cincinnati, Ohio, USA*

[2]*Children's Hospital, Cairo, Egypt*

[3]*Department of Surgery, National Cancer Institute, Cairo University, Cairo, Egypt*

[4]*Weill Cornell Medical College, New York, USA*

[5]*New York Presbyterian Hospital, New York, USA*

[*]**Correspondence to:** Dr. Mohamed Kamel, Department of Surgery, National Cancer Institute, Cairo University, Cairo, Egypt.

Copyright

© 2019 Dr. Mohamed Kamel, *et al.* This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Received: 16 January 2019

Published: 31 January 2019

Keywords: *DNA; Tumor Tissue Genotyping; Cancer Management*

Abstract

Cancer treatment has evolved dramatically over the past decade owing to a better understanding of tumor cell biology and advances in tumor tissue genotyping (TTG). As more targetable genetic mutations are discovered, TTG has become decision-altering in patient management, particularly for cancers with few available traditional treatment options. Moreover, the increasing evidence demonstrating improved oncological outcomes and prognosis with early detection of cancer has made the search for new tumor biomarkers with high sensitivity and specificity an area of particular interest within cancer research.

To maximize the benefit of available treatment options, primary and recurrent tumors should be biopsied to determine newly developed genetic mutations, which may contribute to tumor clonal selection and resistance to therapy.

Circulating tumor DNA (ctDNA), has been extensively studied over the past few years with promising implications for early tumor detection and management. In this review we discuss the role of ctDNA in cancer management.

Introduction

The National Cancer Institute defined biopsy as "the removal of cells or tissues to be examined by a pathologist" [1]. For decades, the identification of specific cell types through tumor tissue sampling (i.e. solid biopsy) has been the mainstay in the diagnosis of malignant tumors. While histopathological assessment of solid tissue biopsies was initially done by a subjective analysis of microscopic cell morphology, the introduction of more advanced staining techniques such as immunohistochemistry significantly improved diagnostic accuracy while reducing interpersonal variability. More recently, a better understanding of tumor cell molecular changes has raised concerns regarding the reliability of solid biopsies' results. In particular, questions arose as to whether an obtained solid biopsy was truly representative of the entire tumor, and whether re-biopsy of tumors with newly-acquired treatment resistance would alter the management strategy. To address these concerns, substantial efforts were made to identify new tumor specific biological markers that would help in the early detection of precancerous lesions and recurrent tumors. Despite the growing evidence that early detection of malignant tumors may improve patients' survival, currently available biomarkers still lack the desirable diagnostic accuracy [2].

The presence of free DNA fragments in the circulation was first reported by the French scientists Mandel and Metais in 1948 [3]. However, the impact of such a discovery on cancer management was not thoroughly investigated until recently, owing to the revolutionary advances in the DNA detection techniques. Numerous studies have since shown that circulating free DNA (cfDNA) could be detected in blood samples from any individual with varying levels. Particularly, pregnancy, inflammation, necrosis, and malignancy were associated with higher detectability of cfDNA [4-8].

The term "liquid biopsy" refers to the ability to detect circulating tumor cells (CTCs) and genetic material (e.g. DNA, RNA, microRNA and exosomes) in the bloodstream [5]. In cancer patients, circulating tumor DNA (ctDNA) has been detected in the blood with tumors as small as 50 million cells [6] and has therefore generated excitement for the profound implications for earlier cancer detection. Despite these great potentials, the application of ctDNA in routine practice is currently met by several challenges. Particularly, as ctDNA exists in minute amounts in blood, the ability to differentiate ctDNA from cfDNA that exists in larger amounts can be challenging [4,7].

Mechanism of ctDNA Shedding

Several hypotheses explaining how tumor DNA enters the circulation have been described. The most plausible theory is that ctDNA is released from necrotic and apoptotic tumor cells [8]. The increase in cell

turnover that accompanies tumor growth overwhelms the ability of phagocytes to clear cellular debris thus permitting the release of cfDNA in the circulation. Another hypothesis suggests that CTCs by themselves may be a source of ctDNA [9].

Virtosomes, a less investigated source of nucleic acid in blood, are DNA-RNA-lipoprotein complexes released from living cells in a more highly regulated manner compared to other forms of cfDNA. Their release is energy dependent and the extracellular environment greatly influences its synthesis and release. Being secreted in lipoprotein complexes, its nucleic acid can only be detected if isolated from these complexes, which can be challenging and costlier. This might imply that nucleic acids sequestered within virtosomes may escape detection by commercially available cfDNA detection kits [10-14]. See figure 1.

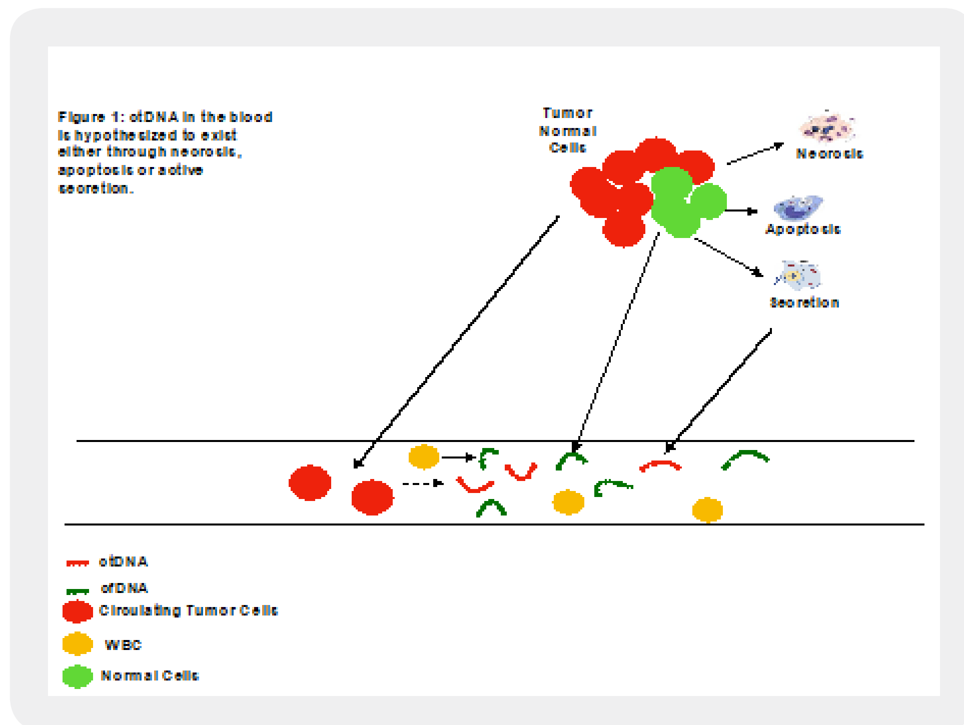

Figure 1

ctDNA Versus cfDNA

ctDNA has the potential to revolutionize early cancer detection compared to the currently used diagnostic modalities (i.e. imaging and biomarkers). However, the average level of cancer ctDNA in the blood is less than 1% of the total cfDNA [4], and thus the ability to differentiate ctDNA from other cfDNA is a major challenge. ctDNA can be differentiated from cfDNA by the presence of somatic mutations that are identical to those in the primary tumor. Commonly detected mutations include single base substitutions, deletions and insertions (e.g. EGFR and KRAS), rearrangements (e.g. EML4-ALK), amplifications (e.g. HER2andMET), and aneuploidies [4,7]. Although on average 80% of solid tumors have mutant genes [15], mutations can only be detected in 0.02% to 0.1% of all DNA assayed as reported by Schwarzenbach *et al*

[16]. Moreover, due to ctDNA high specificity, the fraction of cfDNA that is captured as ctDNA is reported to be as low as 0.01% [17].

Techniques for ctDNA Detection

Researchers have primarily relied on one of two methods for detecting mutations in ctDNA. The first method is to identify mutations in the primary tumor sample and then quantify these mutations in the cfDNA [4]. The other method is to blindly assess cfDNA extracted from plasma for common mutations of significance, independent of the primary tumor mutations [18]. The quantified mutation is then represented as mutant fragments per mililiter [7].

Standardization of pre-analytical steps for blood handling may have a huge impact on optimization of the analysis and ctDNA mutation detection [19]. Qin *et al* experimented using specialized collection tubes (Cell-Free RNA BCT™s) to reduce DNA degradation by nucleases and contamination by white blood cells [20].

Digital polymerase chain reaction (PCR) and beads, emulsion, amplification, and magnetics (BEAMing) [21] or pyrophosphorolysis-activated polymerization (PAP) [22] are next-generation sequencing (NGS) techniques that can identify rare mutant variants in complex mixtures of DNA [23]. They also have the ability of detecting single point mutation, amplifications, rearrangements, and aneuploidy4. This is because they have the necessary bandwidth to detect mutations circulating at low allele frequencies [24].

There are two methods to detect tumor specific rearrangements and chromosomal copy number changes (e.g. amplifications). The first is personalized analysis of rearranged ends (PARE) which uses these alterations for development of tumor biomarkers. The second is digital karyotyping which is a genome-wide method for detection of copy number alterations associated with such chromosomal changes with sensitivity lower than 0.001%. [25,26]. Both approaches can be used in a blind fashion to detect tumor specific rearrangements and amplifications from ctDNA without analyzing the primary tumor [27].

Diehl *et al*, conducted two studies using a BEAMing technique to compare mutations between the primary tumor and ctDNA of colon cancer patients undergoing different modality treatment. They reported that mutations found in primary tumors were consistent with those found in ctDNA and that the sensitivity for stage IV disease was 100.0% [4,28]. But the sensitivity of these techniques still needs improvement for screening and early detection because a ctDNA fraction at or below 0.01% is interpreted as a negative value due to the DNA polymerase error rate [4,29]. This has to be addressed in future techniques utilizing NGS if ctDNA is to be routinely used.

In another study, Chong *et al* evaluated the feasibility of utilizing a targeted DNA sequencing approach with the Ion PGM and AmpliSeq Cancer Panel to detect mutations in 50 cancer-related genes in matched plasma ctDNA and tumor DNA samples from 58 early-stage NSCLC patients. The team reported 89.7% quantifiable cfDNA and 60.3% ctDNA with 50.4% concordance between tumor DNA and ctDNA, sensitivity of 53.8% and specificity of 47.3% [30]. These results are far superior to other commonly used tumor biomarkers.

Despite their promising results, ctDNA detection techniques like Sanger sequencing have their limitations, especially in early stage cancers, as these approaches can be used only in patients with heavy tumor burden [7]. Therefore, these standard approaches were reported to have inferior results compared to other biomarkers like circulating tumor cells [31].

Role of ctDNA in Screening and Diagnosis of Cancer

Diagnostic imaging methods that are currently used in routine practice such as mammography in breast cancer and low dose CT in lung cancer almost always have the same shortcomings. One shortcoming is the subjective and non-specific criteria used to distinguish malignant from benign lesions. Of course, these modalities also subject patients to radiation exposure. Patients may be further exposed to subsequent radiation in instances where a borderline a 'suspicious' lesion requires frequent imaging. Such difficulties are more prominent with smaller lesions [32]. Early stage lung cancer (stage IA) has a 73% 5-year survival rate compared to 24% for stage IIIA. Since only 30% of lung cancers are diagnosed at an early stage [33], ctDNA holds a promise for better outcomes [34].

Using blood biomarkers has even more unreliable results. Carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), carbohydrate antigen (CA125), cytokeratin 19 fragment (CYFRA21-1), and neuron-specific enolase (NSE) are serum biomarkers that have been used for decades in screening and follow up of cancer patients. But these markers are unreliable as other pathologies can cause an increases in their serum levels [32,35].

Currently, diagnosis of most cancers depends solely on a solid biopsy. However, a solid biopsy can be an inconvenient choice for multiple reasons including the relatively longer time needed to obtain and process the specimen as well as the financial cost associated with it. Moreover, biopsies are invasive and pose a risk for complications. According to MD Anderson Cancer Center, adverse event were reported in 17.1% and 1.6% of thoracic and abdominopelvic biopsies, respectively [36].

Another issue, with solid biopsy acquisition is the number of tumor cells obtained and whether it represents the whole cell population or missed sub clones from tumor cells that can tamper with TG results and consequently treatment options. This problem is more evident in advanced tumors which exhibit more heterogeneity [37]. PIK3CA mutations in early stage NSCLC were reported to be four fold in ctDNA compared to tumor DNA, while TP53 and KRAS mutations were found in almost equal proportions [30], emphasizing that ctDNA analysis can give a generalized overview of tumor genome compared to solid biopsy. Moreover, methods used to preserve the biopsy have been questioned due to their potential to affect sequencing accuracy as reported by Luis *et al*, 2014 [7].

Inaccessibility to a tumor was a major concern as with central nervous system (CNS) tumors. There is a possibility of cancer cell dissemination and seeding following standard biopsy procedures. However, CNS tumor-associated mutations can be detected in both the plasma and cerebral spinal fluid of the patients with primary CNS lymphoma [38]. All of these factors have contributed to scientists' enthusiasm of developing ctDNA for use in clinical practice. ctDNA can be found in blood of patients with tumors as small as 50 million cells [6] and, it represents a non-contaminated, fresh source of tumor DNA. Also, ctDNA detection

has very high sensitivity in estimating tumor burden. Even with stage IV disease the level of ctDNA varied with the level of metastases showing less detection of ctDNA fragments with oligo-metastatic disease [28]. In a group of 52 NSCLC patients, cfDNA concentration was found to be highly reflective of tumor burden as the concentration of cfDNA for stage II patients was 14.28ng/ml compared to 4.57ng/ml for stage I tumors (p=0.050) [30]. The wide variance in the level of cfDNA can be used to predict tumor burden in patients but these results needs to be validated in large multicenter randomized trials.

Screening and early detection may be complicated by the fact that a ctDNA fraction at or below 0.01% is interpreted as a negative value due to DNA polymerase error rate. However, other methods that can serve as good screening tools. For instance, the detection of ctDNA methylation has been previously reported to reflect same levels of primary tumor methylation. Although this method lacks specificity compared to the detection of genomic alterations, it is fairly sensitive in earlier disease changes so that it can be used as a good screening tool [39].

The Role of ctDNA in Cancer Management and Prognosis

Evaluation of the tumors' mutational changes that occur with treatment, progression, and recurrence are crucial in cancer management. We can see many studies published over the past decades lacking in reporting the changes in tumor environment that resulted in therapy resisitance [40,41]. Re-biopsying solid tumors may be inconvenient, especially for patients with long treatment courses. However, detecting ctDNA is considered to be minimally invasive and more rapid. Also, it can be assessed at any time in the disease course instead of relying on one time point.

In scenarios where a patient has been treated for a malignancy and is now under follow up or having a stable disease with or without treatment, the oncologist is often faced with the problem that most malignancies do not have a biomarker that is sensitive, specific, and detectable in the blood for long durations18. Commonly used methods of surveillance for early recurrences are computerized tomography (CT) and [18] fluoro-2-deoxyglucose positron emission tomography (PET) scans. Although these methods are considered the standard of care, they fail to provide significant benefit to patient survival [42]. ctDNA can potentially overcome this as it is identified by tumor specific mutations and has shorter half-life (about 2 hours) thus reflecting the current status of disease as accurately as possible [4,43]. Subsequently, it can be used to predict responses and progression early in the course of therapy.

Perhaps one of the most important applications of ctDNA in clinical practice is for surveillance of newly emerging resistance to targeted agents. This can spare patients from numerous drug side effects and ineffective treatment modalities. Imitinib resistance has been well studied in chronic myeloid leukemia patients with documented Philadelphia positive chromosome who initially responded to the drug. The resistance was attributed to acquired mutations in the ABL kinase domain [44]. In lung cancer, there is 50% resistance to gefitinib or erlotinib due to emergence of EGFRT790M variants [45,46]. In this case the cause for resistance to EGFR inhibitors was determined by obtaining biopsies from the tumor but the same results can be obtained by analysis of the ctDNA obtained from the patients [47]. KRAS mutations or MET amplification were attribute to cetuximab and panitumumab resistance48. Misale *et al*, 2012, were able to detect emergent KRAS mutation and subsequent anti-EGFR resistance months before imaging studies

were able to detect treatment failure [48]. Accordingly, ctDNA analysis before, during and after the course of treatment offers a continuous and dynamic view on the tumor genotype. This can surpass current approaches as it can be employed in tailoring future combination anticancer therapy that limits expansion of resistant sub-clones of cancer cells.

Diehl *et al* conducted a study on a group of 20 early stage colorectal cancer patients underwent surgical resection of the tumor for curative intent and positive mutations were determined in the primary tumor of each patient. Post-operative ctDNA was quantified after surgery for each patient. This was followed by several follow ups for up to 5 years. Sixteen patients with detectable levels ctDNA experienced recurrences with one exception. It is worth mentioning that the four patients that had undetectable ctDNA in their first follow up visit experienced no recurrences. First follow up visit was set 13 to 56 days post-surgery which makes ctDNA an early marker that can offer more insight for decision making in adjuvant therapy treatment. Several studies have been conducted in that context, all augmenting the hypothesis that ctDNA can be used as successful marker for minimal residual disease [4,49].

In 85% of diffuse large B-cell lymphoma (DLBCL) cases clonal VDJ sequences are successfully determined in baseline tumor biopsy [50]. Though targeted mutational panels for ctDNA in DLBCL are now being developed, a baseline biopsy is still needed to establish the origin of the tumor [24]. However, pretreatment biopsy quality still remains an issue for ctDNA utilization in DLBCL as a biomarker. Similar to solid tumors, spatial and temporal heterogenicity are concerning in DLBCL. DLBCL show continuous mutational evolvement over time especially with treatment selection [51]. This is more evident in recurrent disease which often exhibits multiple genetically distinct sub clones. Serial ctDNA analysis for mutation allele frequency can reveal newly evolving dominant clones resulting in its potential treatment implications [45,52,53]. This observation precedes CT detection of recurrences by months [50]. Kurtz *et al.*, conducted a study on 75 patients with DLBCL demonstrating that monitoring cell-free ctDNA of VDJ in patients' plasma was more effective than monitoring circulating cells with the same assay [54].

In these studies, it is not clear to us if the practice of giving adjuvant anticancer agents to ctDNA positive subjects actually affects the outcomes. This in itself raises many questions that must be addressed in future trials, including but not limited to the effectiveness of adjuvant treatment in both ctDNA positive and negative subjects and whether we can eliminate adjuvant therapy in ctDNA negative subjects sparing them from drug toxicity and reducing care costs. Another subject if these adjuvant agents do not have the required impact on outcome, what is the future approach we need to adopt to find druggable targets and whether ctDNA can aid in identifying any.

Discussion

Circulating tumor DNA holds the promise of improving patient care. It has proved to be a highly specific biomarker with clear advantages over currently used biomarkers and imaging modalities. Although ctDNA lacks the desired sensitivity when used as marker for smaller tumors, it can still be used as a tool to guide management of late stage cancers where sensitivity is less of an issue. ctDNA utilization may replace solid invasive biopsies, since the higher tumor burden will allow enough ctDNA to be monitored in the circulation and the systemic release from all tumor sites will provide better view of molecular heterogeneity, thus, improving combination therapy tailoring for the patients. ctDNA may also allow for the detection of

response to therapy since ctDNA has an advantage over imaging and tumor markers to predict patient responses to treatment and prognosis in terms of time, specificity and sensitivity. The possibility of rapid, minimally invasive sampling to provide a dynamic, personalized view on tumor genomics holds promise for delivering outstanding results in late-stage patient care. The rapid improvement in NGS will decrease the high costs accompanying the application of this techniques [10,42].

Clinical experience with rare and less frequently encountered tumors suggests that resorting to methods like ctDNA can be beneficial. First, the early detection of the type of response aids in clinical decision to shift to another line of treatment. Second, the specificity of the marker will help in categorizing evolutions in the tumor genomics that emerge with treatment, and thus allowing for skipping years of research due to poor enrolment [55-57].

It might be a while till ctDNA makes it in the area of screening and early detection. But improving the sensitivity of NGS technologies will help their incorporation in patient management plan, from early detection, diagnosis, monitoring treatment response to fine tuning treatment options, thus improving patients' care and reducing the associated costs on many aspects.

Conclusion

ctDNA has the potential to be an ideal biomarker for early cancer detection and post-treatment surveillance. Future advances in cfDNA detection techniques as well as the expected reduction in its cost may further encourage oncologists to adopt this technique.

Bibliography

1. Institute, N. C. National Cancer Institute. NCI Dictionary of Cancer Terms. (2015).

2. Thomas, C. M. & Sweep, C. G. (2001). Serum tumor markers: past, state of the art, and future. *Int J Biol Markers., 16*(2), 73-86.

3. Glazova, M. (1984). Mandel'štam and Dante: The Divine Comedy in Mandel'štam's poetry of the 1930s. *Stud. Sov. Thought., 28*(4), 281-335.

4. Diehl, F., *et al*. Circulating mutant DNA to assess tumor dynamics. *Nat. Med., 14*(9), 985-990 (2008).

5. Bettegowda, C., *et al*. (2014). Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci. Transl. Med., 6*(224), 224ra24.

6. Diaz Jr, L. A., *et al*. (2012). The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. *Nature, 486*(7404), 537.

7. Diaz, L. A. & Bardelli, A. (2014). Liquid biopsies: Genotyping circulating tumor DNA. *J. Clin. Oncol., 32*(6), 579-586.

8. Jahr, S., *et al.* (2001). DNA Fragments in the Blood Plasma of Cancer Patients: Quantitations and Evidence for Their Origin from Apoptotic and Necrotic Cells. *Cancer Res., 61*(4), 1659-1665.

9. Heitzer, E., *et al.* (2013). Establishment of tumor-specific copy number alterations from plasma DNA of patients with cancer. *Int. J. cancer., 133*(2), 346-356.

10. Gaber, O., *et al.* (2018). Virtosome: Is it the Answer to Liquid Biopsy Dilemma? *CPQ Cancer., 1*(3), 1-14.

11. Cataldi, S. & Viola-Magni, M. (2016). Components of the cytosolic and released virtosomes from stimulated and non-stimulated human lymphocytes. *Biochem. Biophys. Reports., 6,* 236-241.

12. Gahan, P. B. & Stroun, M. (2010). The virtosome-a novel cytosolic informative entity and intercellular messenger. *Cell Biochem. Funct., 28*(7), 529-538.

13. Bronkhorst, A. J., *et al.* (2016). Characterization of the cell-free DNA released by cultured cancer cells. *Biochim. Biophys. Acta - Mol. Cell Res., 1863*(1), 157-165.

14. Thierry, A. R., El Messaoudi, S., Gahan, P. B., Anker, P. & Stroun, M. (2016). Origins, structures, and functions of circulating DNA in oncology. *Cancer Metastasis Rev., 35*(3), 347-376.

15. Wood, L. D., *et al.* (2007). The Genomic Landscapes of Human Breast and Colorectal Cancers. *Science, 318*(5853), 1108-1113.

16. Schwarzenbach, H., Hoon, D. S. B. & Pantel, K. (2011). Cell-free nucleic acids as biomarkers in cancer patients. *Nat. Rev. Cancer., 11*(6), 426-437.

17. Bohers, E. *et al.* (2015). Somatic mutations of cell-free circulating DNA detected by Next Generation Sequencing reflect the genetic changes in both Germinal Center B-Cell like and Activated B-Cell like Diffuse Large B-Cell Lymphoma tumors at the time of diagnosis. *Haematologica, 100*(7), e280-4.

18. Forshew, T. *et al.* (2012). Noninvasive Identification and Monitoring of Cancer Mutations by Targeted Deep Sequencing of Plasma DNA. *Sci. Transl. Med., 4*(136), 136ra68.

19. El Messaoudi, S., Rolet, F., Mouliere, F. & Thierry, A. R. (2013). Circulating cell free DNA: Preanalytical considerations. *Clinica Chimica Acta., 424,* 222-230.

20. Qin, J., Williams, T. L. & Fernando, M. (2013). A novel blood collection device stabilizes cell-free RNA in blood during sample shipping and storage. *BMC Res. Notes., 6,* 380.

21. Dressman, D., Yan, H., Traverso, G., Kinzler, K. W. & Vogelstein, B. (2003). Transforming single DNA molecules into fluorescent magnetic particles for detection and enumeration of genetic variations. *Proc. Natl. Acad. Sci. U. S. A., 100*(15), 8817-8822.

22. Liu, Q. & Sommer, S. S. (2000). Pyrophosphorolysis-activated polymerization (PAP): application to allele-specific amplification. *Biotechniques, 29*(5), 1072-1076.

23. Taly, V., *et al*. (2013). Multiplex picodroplet digital PCR to detect KRAS mutations in circulating DNA from the plasma of colorectal cancer patients. *Clin. Chem., 59*(12), 1722-1731.

24. Kurtz, D. M., *et al*. (2015). Dynamic Noninvasive Genomic Monitoring for Outcome Prediction in Diffuse Large B-Cell Lymphoma. *Blood, 126*(23), 130.

25. Leary, R. J., *et al*. (2010). Development of personalized tumor biomarkers using massively parallel sequencing. *Sci. Transl. Med., 2*(20), 20ra14.

26. Wang, T. L., *et al*. (2002). Digital karyotyping. *Proc. Natl. Acad. Sci. U. S. A., 99*(25), 16156-16161.

27. Leary, R. J., *et al*. (2012). Detection of chromosomal alterations in the circulation of cancer patients with whole-genome sequencing. *Sci. Transl. Med., 4*(162), 162ra154.

28. Diehl, F., *et al*. (2005). Detection and quantification of mutations in the plasma of patients with colorectal tumors. *Proc. Natl. Acad. Sci. U. S. A., 102*(45), 16368-16373.

29. Li, M., Diehl, F., Dressman, D., Vogelstein, B. & Kinzler, K. W. (2006). BEAMing up for detection and quantification of rare sequence variants. *Nat. Methods., 3*(2), 95-97.

30. Chen, K. Z., *et al*. (2016). Circulating Tumor DNA Detection in Early-Stage Non-Small Cell Lung Cancer Patients by Targeted Sequencing. *Sci Rep., 6,* 31985.

31. Maheswaran, S., *et al*. (2008). Detection of Mutations in EGFR in Circulating Lung-Cancer Cells. *N. Engl. J. Med., 359*(4), 366-377.

32. Casali, M., Froio, A., Carbonelli, C. & Versari, A. (2013). PET/CT Imaging in Oncology: Exceptions That Prove the Rule. *Case Rep. Oncol. Med., 2013,* 865032.

33. Goldstraw, P., *et al*. (2007). The IASLC Lung Cancer Staging Project: Proposals for the Revision of the TNM Stage Groupings in the Forthcoming (Seventh) Edition of the TNM Classification of Malignant Tumours. *J. Thorac. Oncol., 2*(8), 706-714.

34. Siegel, R. L., Miller, K. D. & Jemal, A. (2015). Cancer statistics, 2015. CA. *Cancer J. Clin., 65*(1), 5-29.

35. Koprowski, H., Herlyn, M., Steplewski, Z. & Sears, H. (1981). Specific antigen in serum of patients with colon carcinoma. *Science, 212*(4490), (1981).

36. Overman, M. J., *et al*. (2013). Use of Research Biopsies in Clinical Trials: Are Risks and Benefits Adequately Discussed? *J Clin Oncol., 31*(1), 17-22.

37. Vogelstein, B., *et al*. (2013). Cancer genome landscapes. *Science, 339*(6127), 1546-1558.

38. Fontanilles, M., *et al*. (2015). Somatic Mutations Detected in Plasma Cell-Free DNA By Targeted Sequencing: Assessment of Liquid Biopsy in Primary Central Nervous System Lymphoma. *Blood, 126*(23), 332.

39. Li, M., *et al*. (2009). Sensitive digital quantification of DNA methylation in clinical samples. *Nat. Biotechnol., 27*(9), 858-863.

40. Eldessouki, I., *et al*. (2018). Small or Non-Small Cell Lung Cancer Based Therapy for Treatment of Large Cell Neuroendocrine Cancer of the Lung? University of Cincinnati Experience. *J. Oncol., 2018,* 1-6.

41. Eldessouki Ihab, Gaber Ola, Riaz Muhammad, K. & Wang Jiang, A. K. N. (2018). Clinical Presentation and Treatment Options for Clear Cell Lung Cancer: University of Cincinnati a Case Series and Literature Review of Clear Cell Lung Cancer. *Asian Pacific Journal of Cancer Prevention, 19*(9), 2373-2376.

42. Thompson, C. A., *et al*. (2014). Utility of routine post-therapy surveillance imaging in diffuse large B-cell lymphoma. *J. Clin. Oncol., 32*(31), 3506-3512.

43. Abdel Karim, N., *et al*. (2018). GNQ-209P Mutation in Metastatic Uveal Melanoma and Treatment Outcome. *Case Rep. Oncol. Med., 2018,* 1-5.

44. Branford, S., *et al*. (2003). Detection of BCR-ABL mutations in patients with CML treated with imatinib is virtually always accompanied by clinical resistance, and mutations in the ATP phosphate-binding loop (P-loop) are associated with a poor prognosis. *Blood, 102*(1), 276-283.

45. Murtaza, M., *et al*. (2013). Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. *Nature, 497*(7447), 108-112.

46. Pao, W., *et al*. (2005). Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med., 2*(3), e73.

47. Taniguchi, K., *et al*. (2011). Quantitative detection of EGFR mutations in circulating tumor DNA derived from lung adenocarcinomas. *Clin. Cancer Res., 17*(24), 7808-7815.

48. Misale, S., *et al*. (2012). Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. *Nature, 486*(7404), 532-536.

49. Mouliere, F., *et al*. (2011). High fragmentation characterizes tumour-derived circulating DNA. *PLoS One, 6*(9), e23418.

50. Roschewski, M., *et al*. (2015). Circulating tumour DNA and CT monitoring in patients with untreated diffuse large B-cell lymphoma: a correlative biomarker study. *Lancet Oncol., 16*(5), 541-549.

51. Roschewski, M., Staudt, L. M. & Wilson, W. H. (2016). Dynamic monitoring of circulating tumor DNA in non-Hodgkin lymphoma. *Blood, 127*(25), 3127-3132.

52. Meador, C. B. & Lovly, C. M. (2015). Liquid biopsies reveal the dynamic nature of resistance mechanisms in solid tumors. *Nat. Med., 21*(7), 663-665.

53. Frenel, J. S., *et al*. (2015). Serial next-generation sequencing of circulating cell-free DNA evaluating tumor clone response to molecularly targeted drug administration. *Clin. Cancer Res., 21*(20), 4586-4596.

54. Kurtz, D. M., *et al*. (2015). Noninvasive monitoring of diffuse large B-cell lymphoma by immunoglobulin high-throughput sequencing. *Blood, 125*(24), 3679-3687.

55. Karim, N. A., *et al*. (2018). Pulmonary sarcomatoid carcinoma: University of Cincinnati experience. *Oncotarget, 9*(3), 4102-4108.

56. Darwish, A., Samra, M., Alsharkawy, N. & Gaber, O. (2016). Adult Biphenotypic Acute Leukemia: The Egyptian National Cancer Institute Experience. *Learning Center,* 134566.

57. Karim, N., *et al*. (2017). A case study in advanced lung cancer patients with vimentin over expression. *Clin. Lab., 63*(10), 1575-1579.