Type of study : Review Article

Liquid Biopsies in Cancer Diagnosis

R.Sharmila[1], Anitha Roy[2], K. Anjaneyulu[3], Lakshmi.T[4]

[1]Research Associate, Dental Research Cell, [2]Associate Professor, Department of Pharmacology, [3]Reader, Department of Conservaive Dentistry and Endodontic, Saveetha Dental College and Hospitals,, Saveetha Institute of Medical and Technical Sciences, Saveetha University,, Chennai-77, India, [4]Associate Professor, Department of Pharmacology, Saveetha Dental College, Saveetha University, Chennai, India

Abstract

Over many years, the concept of precision medicine has dramatically renewed the sphere of clinical oncology; the advent of patient-tailor-made therapies has appreciably stepped forward all measurable outcomes. Liquid biopsy is a modern method that commences unexpected perspectives. It includes the detection and isolation of circulating tumor cells, circulating tumor DNA and exosomes, as a supply of genomic and proteomic records in patients with most cancers. Many technical hurdles had been resolved to newly advanced techniques and subsequent-generation sequencing analyses, permitting a wide software of liquid biopsy in a huge variety of settings. Initially correlated to analysis, liquid biopsy facts at the moment are being studied for cancer analysis, with a bit of luck consisting of screenings, and most significantly for the prediction of reaction or resistance to given remedies. In specific, the identification of particular mutations in genes can be useful resources in therapeutic choices, both in the appropriateness of remedy and within the superior identity of secondary resistance, aiming to early diagnose disease progression. Still, the utility is far from fact however ongoing research is leading the manner to a new era in oncology. The main aim of this study is to assess the importance of liquid biopsy in cancer diagnosis.

Keywords*: Liquid biopsy, cancer, exosomes, circulating tumor cells, circulating tumor DNA, oncology.*

Introduction

Liquid biopsy is the sampling and analysis of nonsolid biological tissue, primarily blood[1]. It is a test done on a sample of blood to look for cancer cells form a tumor that might be circulating in blood or pieces of DNA from tumor cells that are in the blood. It is used to assist cancers at an early stage [2]. It will also be used to assist plan treatment or discover how the treatment is running or if cancer has come back [1]. Being able to take more than one sample of blood over time, may additionally assist medical doctors to apprehend what kind of molecular changes are taking place in a tumor [3].

Corresponding Author:
Anitha Roy,
Associate Professor, Department of Pharmacology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-77, India, Email : anitharoy@saveetha.com

Liquid biopsy is an easy and non-invasive opportunity for surgical biopsies which allows doctors to find information about tumors via a simple blood pattern[4]. Traces of cancer's DNA inside the blood can deliver clues about which remedies are most possible to work for affected people[2]. A liquid biopsy takes a look that can locate epidermal increase component receptor gene mutations, which occur in 10-35 % sufferers with non-small lung cancers, will assist to pick out the proper treatment for the affected person at the right time[5]. Much of early research on liquid biopsies has been in lung, breast, and prostate cancers however this generation is predicted to affect all forms of cancer[3]. Oxidative stress plays an important role in the pathogenesis of various diseases especially in chronic liver disease[6]. Hepatic fibrosis is considered to be the major issue in liver diseases[7]. The non-invasive nature of liquid biopsies, which require 5 milliliters of blood, means they're a lot easier to tolerate and the method is quicker than a surgical biopsy [8].

Tolerability and comfort are the main raise for sufferers[3]. The biggest advantage lies inside the ability of liquid biopsies to locate sickness development or remedy resistance long earlier than it would cause clinical signs and symptoms or seen on imaging scans [4,9]. Most cancers have a couple of genetic mutations and they'll now not have the equal ones in all parts of the most cancers[1]. The tissue samples eliminated for biopsy won't show all mutations whereas liquid biopsies offer a stepped forward danger of detecting those genetic modifications[5]"Liquid biopsies might be a -changer in cancer diagnosis," stated Miro Venturi."In terms of affected person acceptability and sickness control, the blessings of non-invasive, quick, and without difficulty repeatable exams are clear[10]. And inside the long term, liquid biopsies might also in the long run be used to capture symptoms of most cancers early, earlier than symptoms arise[11]. This could make a big distinction in the way we recognize and deal with cancer[12].There is increased mortality with cancer because of the toxic side effects of cancer chemotherapy and radiation therapy[13]. So medicinal plants are used as a substitute for conventional drugs because of their increasing demand for plant-based natural products. *Caralluma fimbriata* which is found in dry regions of Tamil Nadu has been known for its anticancer activities with no adverse effects [13,14]. It is also used against human colon cancer cells[13]. *Azadirachta indica* also has various medicinal properties[15]. Recent studies use herbal medicine as potent anticancer drug candidates[16]. Coumarin plays an important role in the drug discovery process and is also used to treat cancer[17]. *Acacia catechu* is known for its hypoglycemic, free radical scavenging and antioxidant properties. In this study, it has been used against SCC-25 human oral squamous carcinoma cell line[18].

Liquid Biopsy- Investigating Tumor

Cancer is one of the main causes of death, with 9.6 million cancer deaths in the world[3]. Hepatocellular carcinoma is the second most common cancer and is associated with increased mortality in the last two decades[19]. The improvement of technology has led to the sphere of precision oncology, which accommodates treatment regimens to the molecular traits of every patient's tumor [20]. The modern widespread genetic profiling of tumors commonly involves the usage of tissue biopsies. Despite their invasive nature, tissue biopsies are associated with many barriers, inclusive of patient chance, sample coaching, sensitivity and accuracy, procedural expenses, and invasiveness[21]. This makes the manner incompatible for clinical longitudinal tracking [22]. Furthermore, a significant dilemma of tissue biopsies is that they fail to seize intratumoral and anti-metastatic genetic heterogeneity, impacting the accuracy[23].

Liquid biopsies have an excellent capacity to triumph over these existing sampling barriers. Such biopsies comprise the sampling and analysis of liquid organic sources, usually blood, for cancer analysis, screening, and prognosis[12]. The 'tumor circle', described because the subset of circulating additives is derived from cancer tissue and may be immediately or circuitously used as a source of most cancers biomarkers in liquid biopsies[24] . These components encompass circulating tumor proteins, circulating tumor nucleic acids (ctDNA and ctRNA), CTCs, EVs, and TEPs [23]. Liquid biopsies present numerous advantages over conventional tissue biopsies), and technological improvements in sample isolation[25]. The first critical milestone in this subject was reached in 2016 with the FDA approval of the first companion diagnostic test for lung cancer primarily based on the ctDNA content of a liquid biopsy.

OTHER METHODS OF CANCER DIAGNOSIS

There are various methods of a cancer diagnosis. Cancer detection often involves radiological imaging. Imaging is used to check the spread of cancer and prognosis of treatment, and to monitor cancer oncological imaging is more varied and accurate[26]. Different imaging techniques aim to find the most suitable treatment option for each patient. Imaging techniques are often used in combination to obtain sufficient information[27]. These include computed tomography, Magnetic Resonance Imaging, Ultrasonic endoscopic examination, mammography, and isotopic diagnosis[28].

Laboratory tests are carried out when it is suspected that a patient has cancer. Normally blood samples are taken for monitoring blood counting[26]. Genetic testing can be useful in connection with certain cases of cancer [12]. A final diagnosis of cancer is based on an examination of tissues or cells under microscopic by a pathologist[12].

CIRCULATING TUMOR DNA

CtDNA comprises the fraction of circulating cellular- DNA originating from cancer cells. These consist of brief nucleosome-related fragments and longer fragments encapsulated inside EVs [3]. The mechanism of ctDNA encompasses apoptosis, necrosis, lysis of CTCs, and lively secretion from the tumor[29]. The proof of the suitability of ctDNA as a cancer biomarker came with the identity of KRAS gene mutations in ctDNA from the blood of patients with pancreatic cancer[1].

Both qualitative and quantitative data may be acquired from ctDNA evaluation. Quantitative information may be received from the measurement of the mutant allele fraction and is a reflection of the tumor[30]. It finds application inside the detection of minimal residual ailment (MRD) and occult metastases and within the monitoring of treatment response and therapeutic effectiveness [2]), The detection of ctDNA after treatment is an excessive-sensitivity and excessive-specificity predictor of relapse. Qualitative records can be sourced via the profiling of mutations, amplifications, deletions, and translocations in ctDNA allowing the identification of genetic alterations associated with the reaction, as a result assisting selection-making for customized control [30,31]. Other qualitative data obtained through ctDNA evaluation includes an assessment of methylation popularity[29].

CIRCULATING TUMOR CELLS

CTCs are tumor cells that are indifferent from one tumor and can be found in the peripheral blood of sufferers[32]. Their presence is thought to be fundamental to the development of metastasis. CTCs present systemically via energetic intravasation, with the epithelial-to-mesenchymal transition as a fundamental step or through passive shedding from the primary tumor[33]. This latter mechanism is supported through the presence of CTC aggregates or circulating tumor microemboli inside the blood[32].

The records acquired from CTCs are quantitative as well as phenotypic through single-cell genomics or proteomic profiling[34].CTCs have superpotential as tools for the analysis, tracking, prognosis, and prediction of reaction to remedy, and also for the discovery of novel drug objectives. The best records available from CTCs are their variety, which is a prognostic predictor for many cancers, which includes metastatic breast, colon, and prostate cancer[35]. The power of CTC counts as a criterion for the choice of first-line treatment in metastatic breast cancers is currently being investigated[36].

Despite numerous analytical systems and technology to be had for CTC analysis, their translation into medical practice is restricted by means of their isolation from the blood[37]. Challenges consist of their extreme rarity, fragility, and physical and phenotypic heterogeneity[30]. Each of these options has advantages and drawbacks, and best their mixture can support a comprehensive characterization[38].

EXTRACELLULAR VESICLES

Extracellular vesicles are membranous particles released from all cellular types underneath physiological and pathological conditions, in addition to following distinct forms of stimuli, inclusive of proteases, ADP, thrombin, inflammatory cytokines, increase factors, biomechanical shear and pressure inducers, and apoptotic indicators [3,5]. They can be observed in almost every physical fluid, especially blood[3]. EVs had been diagnosed as essential mediators of intercellular communication, regulating and collaborating in a plethora of physiological and pathological procedures, such as cancer. Based on their biogenesis, content, and secretory pathways, EVs may be divided into two large classes: exosomes and microvesicles [5].

The suitability of EVs as cancer biomarkers lies within the reality that the molecular cargoes they create can be considered a cell of origin[39]. The advantages of EVs are many [22]. EVs are usually produced and launched in abundant portions and in greater amounts compared with CTCs[40]. Similar to ctDNA and CTCs, EVs can be a supply of quantitative and qualitative information. Quantitative facts comprising EV can detect the presence of malignant sickness and tumor burden[41]. For instance, circulating exosome tiers are elevated in breast and pancreatic cancer and the number of circulating microparticles is higher in patients with a couple of myelomas as compared with healthful people[42].

SEQUENCING TECHNIQUES

Next-Generation Sequencing

There are various sequencing techniques in liquid biopsy. NGS has emerged beyond the decade as a primary method for sequencing DNA and acquiring genetic statistics[25]. NGS is primarily based on the analysis of several hundreds of thousands of DNA sequences in parallel observed by either series alignment to a reference genome. Despite its excessive sensitivity and specificity, NGS indicates a random error charge between 0.1% and 1% depending on the carried out platform making the detection of ctDNA through uncommon mutations in the general ctDNA[43,44]. According to this remark, many protocols have been changed to enhance and extend the detection of uncommon mutations[25].

PCR based methods

PCR represents a rapid and cheap approach for the amplification of nucleic acid. Polymerase Chain Reaction is used to make billions of copies of specific DNA samples.PCR can determine gene duplication or deletion[25]. Furthermore, melting curve analysis immediately after PCR can identify small mutations, down to single changes. These techniques are becoming easier and faster and can be multiplexed[45]. Real-time PCR methods are favorable options for the analysis of cancer markers[43]. PCR looks for changes in genes or chromosomes, which may help find and diagnose a genetic condition or disease such as cancer [43]. The AS-PCR is commonly used in medical settings to stumble on single nucleotide variation (SNV) or small insertion/deletion in formalin-constant, paraffin-embedded tumor tissues. Overall, PCR based assays is a promising device for detecting mutations as a low-cost powerful may be feasible in ordinary medical exercise[46].

Methylation Sequencing

DNA methylation plays an important role in regulating normal development and carcinogenesis. The current understanding of biological roles of DNA methylation is limited to its role in the regulation of gene transcription, genomic imprinting, X chromosome inactivation[44]. In the past 2 decades, a large number of changes have been identified[47]. These alterations fall into two main categories, namely hypermethylation of tumor suppressor genes and hypomethylation of oncogenes respectively [44]. The development of DNA methylation markers for cancer detection holds the promise of being accurate, sensitive, cost-effective for risk assessment, early diagnosis, and prognosis.[43]

ADVANTAGES

Liquid biopsy has numerous advantages. It is substantially less invasive while compared to tissue biopsy. Test results are generally available a great deal earlier than standard tissue biopsy [48]. Test results are generally available a great deal earlier than standard tissue biopsy[21] . Using a liquid biopsy, it is feasible to make an early diagnosis. Liquid biopsy can be used to estimate a threat for metastatic relapse progression. Liquid biopsy may additionally permit for stratification and real-time monitoring of treatment options[28]. It can be able to higher perceive therapeutic goals. The ease and frequency of a liquid biopsy test give bones over tissue biopsy [4]. The test may be easily repeated and used often as essential to monitor a patient ‘s development. Liquid biopsy is commonly much less expensive to carry out than tissue biopsy [3].

CLINICAL APPLICATIONS

Initial Diagnosis

Liquid biopsy at initial diagnosis may be beneficial for prognostications[12]. Another capacity benefit of liquid biopsy over strong tumor biopsy is that, is unresectable cancers [21,49]. There can be inadequate tissue available form this aspirate of DNA sequencing and liquid biopsy has a non-invasive supply of DNA available. [28]Regardless it might be imprudent to use mutation detected in liquid biopsies for choosing the first-line remedy unless the frequency of a mutant allele is excessive enough to warrant the entire self-being that mutation is likely to be derived from the tumor itself[21].

After Surgery

Liquid biopsy taken after surgical treatment is promising in this context [1]. There is already research displaying that sufferers who have circulating tumor DNA or CTCs following surgery are likely to relapse. In the present state, the sensitivity for detecting disease is far from 100%. A negative test should therefore be considered as another feature [50].

After additional therapies

Liquid biopsy is able to detect easily recurrence prior to tumors becoming radiographically or clinically

apparent, potentially giving clinicians a larger window of opportunity during which treatment regimen could be altered [28]. Once a patient relapses, a liquid biopsy may reveal new mutations not present in the primary tumor that could guide choice for second-line therapy[51]

Screening the cancer

Using liquid biopsies before cancer is clinically detected is discussed last because it is the most difficult application, but also has the greatest potential to reduce morbidity and mortality from cancer[52]. Cost and specificity are less of an issue, sensitivity is more important, as patients untreated on the basis of false-negative tests are likely to die[8]. Much controversy around screening tests is based on relatively low positive predictive values [2]. A related problem is an overdiagnosis, detection of cancers that are indolent and never would cause morbidity or mortality if they remain undetected. [1]

LIMITATION

Liquid biopsy is not considered a popular procedure although there is increasing use of these assays, tissue biopsy remains widespread for affirmation and analysis of sickness, such as numerous cancers and for detection of characteristics of sickness.[32] At present liquid, the biopsy is not used as a substitute for tissue biopsy.[53] More assistance for scientific utility is needed. There is presently, no longer a widespread usage of liquid biopsy taking a loop in clinical networks. More variation in scientific trials is required on the cost of liquid biopsies in medical Settings to support the clinical utility of tests [22]. The studies are needed to assess the test's accuracy, and its ability to identify various tumor types. Test sensitivity challenges still exist[52]. It is also given that circulating tumor cells or DNA are relatively rare compared to the number of hematological molecules found in blood samples, there are challenges to test 's detection ability.[44]

THE FUTURE SCOPE

During the past decade, liquid biopsy has received tremendous attention[28]. But yet the technique is not a standard tool in the clinical oncologist arsenal [54]. One often misunderstood, but critical point is that screening tests do not detect cancers that are very early[8]. All they do is detect cancer earlier than they could be detected. Therapeutics require biomarkers, to stratify responders from non-responders, thereby treating patients most likely to benefit and preventing unnecessary harm to unnecessary patients[3]. Liquid biopsy is likely to provide such markers for any type of cancer[1]. The method of balancing current, intolerable under diagnosis, with potential overdiagnosis is a challenge that further research will hopefully solve. However, a solution to this problem will be possible if reliable early diagnostic tests for major cancer are developed and used[55]. Nanotechnology has also emerged as a promising tool in the treatment and management of cancers[56]. Nanotechnology is also known for its role in target-specific delivery of drugs[57]. Selenium nanoparticles have a vital prospect in the field of medicine and are used as anti-cancer agents [58].Zinc oxide nanoparticles synthesized from *Mangifera indica* has been known for its antioxidant activity and cytotoxic effects on lung cancer[59]. Antibacterial properties of nanoparticles were evaluated[60]. Green synthetic methods of nanoparticles are simple, non-invasive, and eco-friendly [61]. Further studies can be done to evaluate the importance of nanotechnology in the diagnosis and treatment of cancer. With rapidly advancing technology and with accelerating interest in liquid biopsy from both academia and industry, looking forward to the day when liquid biopsy that detects cancer becomes a routine part of preventive medicine [54].

Conclusion

Liquid biopsy has a powerful role in helping patients to get the right treatment. Molecular analysis of cancer is required to optimize patient treatment. New methods such as next-generation show immense promise for the future. Liquid biopsy is coming of age and will change practice, it will enable oncologists to use drugs intelligently to combat the change in individual cancer as they happen the elegant and powerful technique, fulfill the promise of becoming rapid, reliable, and non-invasive decision-making tools.

Acknowledgement: The authors like to thank the editors and authors of the journal, which was the source for the scientific compilation of this review article.

Conflict of Interest : No potential conflict of interest relevant to this article was reported.

Source of Funding : Self

Ethical Clearance: Not Required

References

1. Palmirotta R, Lovero D, Cafforio P, Felici C, Mannavola F, Pellè E, et al. Liquid biopsy of cancer: a multimodal diagnostic tool in clinical oncology. Ther Adv Med Oncol. 2018 Aug 29;10:1758835918794630.
2. Alix-Panabières C, Pantel K. Circulating tumor cells: liquid biopsy of cancer. Clin Chem. 2013 Jan;59(1):110–8.
3. De Rubis G, Rajeev Krishnan S, Bebawy M. Liquid Biopsies in Cancer Diagnosis, Monitoring, and Prognosis. Trends Pharmacol Sci. 2019 Mar;40(3):172–86.
4. Strumfa I, Gardovskis J. Introductory Chapter: Liquid Biopsy — A Promising Technology of the Future [Internet]. Liquid Biopsy. 2019. Available from: http://dx.doi.org/10.5772/intechopen.86918
5. Sumazaki M, Ueda K. Liquid Biopsy Diagnostics Using Extracellular Vesicles [Internet]. Biomarkers in Cancer Therapy. 2019. p. 3–10. Available from: http://dx.doi.org/10.1007/978-981-13-7295-7_1
6. Ezhilarasan D. Oxidative stress is bane in chronic liver diseases: Clinical and experimental perspective. Arab J Gastroenterol. 2018 Jun;19(2):56–64.
7. Ezhilarasan D, Sokal E, Najimi M. Hepatic fibrosis: It is time to go with hepatic stellate cell-specific therapeutic targets. Hepatobiliary Pancreat Dis Int. 2018 Jun;17(3):192–7.
8. Tanos R, Thierry AR. Clinical relevance of liquid biopsy for cancer screening [Internet]. Vol. 7, Translational Cancer Research. 2018. p. S105–29. Available from: http://dx.doi.org/10.21037/tcr.2018.01.31
9. Paeglis A, Strumfs B, Mezale D, Fridrihsone I. A Review on Machine Learning and Deep Learning Techniques Applied to Liquid Biopsy [Internet]. Liquid Biopsy. 2019. Available from: http://dx.doi.org/10.5772/intechopen.79404
10. Incorvaia L, Castiglia M, Perez A, Massihnia D, Caruso S, Altintas S, et al. Liquid Biopsy in Breast Cancer [Internet]. Current Clinical Pathology. 2017. p. 77–84. Available from: http://dx.doi.org/10.1007/978-3-319-55661-1_9
11. Vrabel D, Souckova A, Sedlarikova L, Sevcikova S. Liquid Biopsies in Multiple Myeloma [Internet]. Liquid Biopsy. 2019. Available from: http://dx.doi.org/10.5772/intechopen.78630
12. Russo A, Giordano A, Rolfo C. Liquid Biopsy in Cancer Patients: The Hand Lens for Tumor Evolution. Humana Press; 2017. 214 p.
13. Ashwini S, Ezhilarasan D, Anitha R. Cytotoxic effect of Caralluma fimbriata against human colon cancer cells. Pharmacognosy Journal [Internet]. 2017;9(2). Available from: https://www.phcogj.com/article/252
14. Ashwini S, Anitha R. Antihyperglycemic Activity of Caralluma fimbriata: An In vitro Approach. Pharmacogn Mag. 2017 Oct;13(Suppl 3):S499–504.
15. Lakshmi T, Krishnan V, Rajendran R, Madhusudhanan N. Azadirachta indica : A herbal panacea in dentistry - An update [Internet]. Vol. 9, Pharmacognosy Reviews. 2015. p. 41. Available from: http://dx.doi.org/10.4103/0973-7847.156337
16. Lakshmi T, Ezhilarasan D, Vijayaragavan R, Bhullar SK, Rajendran R. Acacia catechu ethanolic bark extract induces apoptosis in human oral squamous carcinoma cells. J Adv Pharm Technol Res. 2017 Oct;8(4):143–9.
17. Perumalsamy H, Sankarapandian K, Veerappan K, Natarajan S, Kandaswamy N, Thangavelu L, et al. In silico and in vitro analysis of coumarin derivative induced anticancer effects by undergoing intrinsic pathway mediated apoptosis in human stomach cancer. Phytomedicine. 2018 Jul 15;46:119–30.
18. Lakshmi T, Ezhilarasan D, Nagaich U, Vijayaragavan R. Acacia catechu Ethanolic Seed Extract Triggers Apoptosis of SCC-25 Cells. Pharmacogn Mag. 2017 Oct;13(Suppl 3):S405–11.
19. Gheena S, Ezhilarasan D. Syringic acid triggers reactive oxygen species–mediated cytotoxicity in HepG2 cells [Internet]. Vol. 38, Human & Experimental Toxicology. 2019. p. 694–702. Available from: http://dx.doi.org/10.1177/0960327119839173
20. Mantyh PW. Cancer pain and its impact on diagnosis, survival and quality of life. Nat Rev Neurosci. 2006 Oct;7(10):797–809.
21. Arancio W, Belmonte B, Castiglia M, Di Napoli A, Tripodo C. Tissue Versus Liquid Biopsy: Opposite or Complementary? [Internet]. Current Clinical

Pathology. 2017. p. 41–9. Available from: http://dx.doi.org/10.1007/978-3-319-55661-1_4

22. Fernández-Lázaro D, García Hernández JL, García AC, Córdova Martínez A, Mielgo-Ayuso J, Cruz-Hernández JJ. Liquid Biopsy as Novel Tool in Precision Medicine: Origins, Properties, Identification and Clinical Perspective of Cancer's Biomarkers. Diagnostics (Basel) [Internet]. 2020 Apr 13;10(4). Available from: http://dx.doi.org/10.3390/diagnostics10040215
23. Matsutani A, Udagawa C, Matsunaga Y, Nakamura S, Zembutsu H. Liquid biopsy for the detection of clinical biomarkers in early breast cancer: new insights and challenges. Pharmacogenomics. 2020 Apr;21(5):359–67.
24. Scalia P, Williams SJ, Russo A, Giordano A. Actionable Molecular Targets in Cancer Liquid Biopsy [Internet]. Current Clinical Pathology. 2017. p. 71–6. Available from: http://dx.doi.org/10.1007/978-3-319-55661-1_8
25. Saad MM, Gavrilla M, Byron K, Deam D. Clinical cancer multi-gene mutation profiling of solid tumours based on next-generation sequencing (NGS) [Internet]. Vol. 48, Pathology. 2016. p. S60. Available from: http://dx.doi.org/10.1016/j.pathol.2015.12.147
26. Manne U, Srivastava RG, Srivastava S. Keynote review: Recent advances in biomarkers for cancer diagnosis and treatment. Drug Discov Today [Internet]. 2005; Available from: https://www.sciencedirect.com/science/article/pii/S1359644605034872
27. Croce C, Calin G. Compositions and methods for cancer diagnosis and therapy [Internet]. US Patent. 20040152112:A1, 2004 [cited 2020 Jun 10]. Available from: https://patentimages.storage.googleapis.com/5e/68/af/3167acbdfde20f/US20040152112A1.pdf
28. Anderson DJ, Anderson RG, Moug SJ, Baker MJ. Liquid biopsy for cancer diagnosis using vibrational spectroscopy: systematic review. BJS Open [Internet]. 2020 May 19; Available from: http://dx.doi.org/10.1002/bjs5.50289
29. Institute NC, National Cancer Institute. Circulating Tumor-Derived DNA [Internet]. Definitions. 2020. Available from: http://dx.doi.org/10.32388/baazv7
30. Alix-Panabieres C. Circulating Tumor Cells: Finding Rare Events for A Huge Knowledge of Cancer Dissemination. MDPI; 2020. 366 p.
31. Hu Y, Ying HU. Advance in human free circulating DNA [Internet]. Vol. 30, Hereditas (Beijing). 2008. p. 815–20. Available from: http://dx.doi.org/10.3724/sp.j.1005.2008.00815
32. Shaw J. Liquid biopsies: An introduction to circulating tumour cells and ctDNA [Internet]. Vol. 50, Pathology. 2018. p. S30. Available from: http://dx.doi.org/10.1016/j.pathol.2017.12.069
33. Kulasinghe A, Perry C, Jovanovic L, Nelson C, Punyadeera C. Circulating tumour cells in metastatic head and neck cancers [Internet]. Vol. 136, International Journal of Cancer. 2015. p. 2515–23. Available from: http://dx.doi.org/10.1002/ijc.29108
34. Pantel K, Alix-Panabières C. Circulating tumour cells in cancer patients: challenges and perspectives. Trends Mol Med. 2010 Sep;16(9):398–406.
35. Goh KY, Lim W-T. Profiling Circulating Tumour Cells for Clinical Applications [Internet]. Liquid Biopsy. 2019. Available from: http://dx.doi.org/10.5772/intechopen.79228
36. Alix-Panabières C, Pantel K. Clinical Applications of Circulating Tumor Cells and Circulating Tumor DNA as Liquid Biopsy. Cancer Discov. 2016 May;6(5):479–91.
37. Christou N, Meyer J, Popeskou S, David V, Toso C, Buchs N, et al. Circulating Tumour Cells, Circulating Tumour DNA and Circulating Tumour miRNA in Blood Assays in the Different Steps of Colorectal Cancer Management, a Review of the Evidence in 2019 [Internet]. Vol. 2019, BioMed Research International. 2019. p. 1–11. Available from: http://dx.doi.org/10.1155/2019/5953036
38. Hugh Fan Z. Circulating Tumor Cells: Isolation and Analysis. John Wiley & Sons; 2016. 464 p.
39. Rayyan M, Zheutlin A, Byrd JB. Clinical research using extracellular vesicles: insights from the International Society for Extracellular Vesicles 2018 Annual Meeting [Internet]. Vol. 7, Journal of Extracellular Vesicles. 2018. p. 1535744. Available from: http://dx.doi.org/10.1080/20013078.2018.1535744
40. Exosomes as Liquid Biopsy: A Review. IJSR. 2016 Jun 5;5(6):1858–9.
41. Lötvall J, Rajendran L, Gho Y-S, Thery C, Wauben M, Raposo G, et al. The launch of Journal of Extracellular Vesicles (JEV), the official journal of

the International Society for Extracellular Vesicles – about microvesicles, exosomes, ectosomes and other extracellular vesicles [Internet]. Vol. 1, Journal of Extracellular Vesicles. 2012. p. 18514. Available from: http://dx.doi.org/10.3402/jev.v1i0.18514

42. Roy S, Hochberg FH, Jones PS. Extracellular vesicles: the growth as diagnostics and therapeutics; a survey [Internet]. Vol. 7, Journal of Extracellular Vesicles. 2018. p. 1438720. Available from: http://dx.doi.org/10.1080/20013078.2018.1438720
43. Malapelle U, Pisapia P, Rocco D, Smeraglio R, di Spirito M, Bellevicine C, et al. Next generation sequencing techniques in liquid biopsy: focus on non-small cell lung cancer patients. Transl Lung Cancer Res. 2016 Oct;5(5):505–10.
44. Xu H. Differential Methylation Analysis with Next-Generation Sequencing [Internet]. Next Generation Sequencing in Cancer Research, Volume 2. 2015. p. 229–38. Available from: http://dx.doi.org/10.1007/978-3-319-15811-2_14
45. Wj A, Ansorge WJ. Next Generation DNA Sequencing (II): Techniques, Applications [Internet]. Vol. 01, Journal of Next Generation Sequencing & Applications. 2015. Available from: http://dx.doi.org/10.4172/2469-9853.s1-005
46. Singh RS. Recent advances in DNA sequencing techniques [Internet]. 2013. Available from: http://dx.doi.org/10.1063/1.4812627
47. Research CM, Case Medical Research. Liquid Biopsy Using Methylation Sequencing for Lung Cancer [Internet]. Case Medical Research. 2020. Available from: http://dx.doi.org/10.31525/ct1-nct04253509
48. Miyanaga A, Masuda M, Yamada T. Biomarkers of Lung Cancer: Liquid Biopsy Comes of Age [Internet]. Biomarkers in Cancer Therapy. 2019. p. 105–13. Available from: http://dx.doi.org/10.1007/978-981-13-7295-7_10
49. Cescon DW, Bratman SV, Chan SM, Siu LL. Circulating tumor DNA and liquid biopsy in oncology. Nature Cancer. 2020 Mar 1;1(3):276–90.
50. Rolfo C, Castiglia M, Hong D, Alessandro R, Mertens I, Baggerman G, et al. Liquid biopsies in lung cancer: the new ambrosia of researchers. Biochim Biophys Acta. 2014 Dec;1846(2):539–46.
51. Wang J, Chang S, Li G, Sun Y. Application of liquid biopsy in precision medicine: opportunities and challenges. Front Med. 2017 Dec;11(4):522–7.
52. Brock G, Castellanos-Rizaldos E, Hu L. Liquid biopsy for cancer screening, patient stratification and monitoring. Transl Cancer Res [Internet]. 2015; Available from: https://www.researchgate.net/profile/Elena_Castellanos/publication/301633860_Liquid_biopsy_for_cancer_screening_patient_stratification_and_monitoring/links/5ad65ce6458515c60f5694fa/Liquid-biopsy-for-cancer-screening-patient-stratification-and-monitoring.pdf
53. Elazezy M, Joosse SA. Techniques of using circulating tumor DNA as a liquid biopsy component in cancer management. Comput Struct Biotechnol J. 2018 Oct 9;16:370–8.
54. Zhang Z, Ramnath N, Nagrath S. Current Status of CTCs as Liquid Biopsy in Lung Cancer and Future Directions. Front Oncol. 2015 Sep 30;5:209.
55. Adiga R. Liquid Biopsy: Future in Diagnostic Medicine [Internet]. Vol. 1, International Journal of Biosensors & Bioelectronics. 2016. Available from: http://dx.doi.org/10.15406/ijbsbe.2016.01.00003
56. Sharma P, Mehta M, Dhanjal DS, Kaur S, Gupta G, Singh H, et al. Emerging trends in the novel drug delivery approaches for the treatment of lung cancer. Chem Biol Interact. 2019 Aug 25;309:108720.
57. Mehta M, Deeksha, Tewari D, Gupta G, Awasthi R, Singh H, et al. Oligonucleotide therapy: An emerging focus area for drug delivery in chronic inflammatory respiratory diseases. Chem Biol Interact. 2019 Aug 1;308:206–15.
58. Menon S, Ks SD, R S, S R, S VK. Selenium nanoparticles: A potent chemotherapeutic agent and an elucidation of its mechanism. Colloids Surf B Biointerfaces. 2018 Oct 1;170:280–92.
59. Rajeshkumar S, Kumar SV, Ramaiah A, Agarwal H, Lakshmi T, Roopan SM. Biosynthesis of zinc oxide nanoparticles usingMangifera indica leaves and evaluation of their antioxidant and cytotoxic properties in lung cancer (A549) cells. Enzyme Microb Technol. 2018 Oct;117:91–5.
60. Karthiga P, Rajeshkumar S, Annadurai G. Mechanism of Larvicidal Activity of Antimicrobial Silver Nanoparticles Synthesized Using Garcinia

mangostana Bark Extract. J Cluster Sci. 2018 Nov 1;29(6):1233–41.

61. Rajeshkumar S, Agarwal H, Kumar SV, Lakshmi T. Brassica oleracea mediated synthesis of zinc oxide nanoparticles and its antibacterial activity against pathogenic bacteria. Asian J Chem. 2018;30(12):2711–5.