Liquid Biopsy: Novel Progressions and Comparisons

Lavanya Gupta[1], Nicole Klatt[1], Humayd Zameer[1], Sushruta Surappa[2]

1 - University of California, Berkeley, 2 - Canary Center at Stanford for Cancer Early Detection

ABSTRACT

Liquid biopsy technology has evolved into a promising, minimally invasive clinical tool for cancer diagnosis and oncology research. Liquid biopsy is a broad term referring to the testing of bodily fluids: spinal fluid, sweat, urine, and most commonly blood as it is materially dense; it is an analysis technique that detects various biomarkers, including common biomarkers such as circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), and exosomes (Fig. 1). There is particular interest in how effective early cancer diagnosis with liquid biopsies are, and its accuracy in early diagnosis prior to other prominent cancer testing/screenings. After studying a bibliometric review, we identified hot spot topics to focus on and wrote this non-systematic review article regarding new details about common biomarkers (ctDNA, exosomes, and CTC), novel technology, and recent tissue vs. liquid biopsy comparisons (non-small cell lung cancer and glioblastoma). (Fig. 2) More specifically, our study focuses on particular cancers and associated novel progressions in clinical studies that utilize liquid biopsies. This literature review covers updated findings from other reviews and clinical studies centered on recent advances in standardization, development, and application of novel high-throughput technology. This study also compares tissue biopsies—a standard cancer diagnostic technique—and its complementary role in early cancer detection with liquid biopsies. Additionally, this paper explores topical challenges in liquid biopsy specificity, efficacy, and cost-efficiency in regards to personalized cancer diagnosis and treatment.

Introduction

Cancer is the second leading cause of death worldwide[39]. Liquid biopsy is a powerful method contrasting invasive, traditional biopsy methods. Based on biological fluid samples and specific biomarker targets, information on DNA mutations, epigenetic mutations, gene overexpression, and more can be detected through liquid biopsies. Thus, development of liquid biopsy techniques can lead to further innovations in early-detection and monitoring of primary and metastatic tumors.

Types of Biomarker Targets

The biomarkers of ctDNA, exosomes, and CTCs have each been commonly used in liquid biopsy. However, recent findings within each type of biomarker warrants a more detailed inspection regarding their benefits and drawbacks for each of the three biomarkers mentioned.

ctDNA: ctDNA is a subset of cell-free nucleic acids in the blood (cfDNA)[1]. Although active release of DNA from live tumor cells is less understood, it is believed that most ctDNA is sourced from various cell death pathways due to tumor cell shedding ("apoptosis, necrosis, ferroptosis, pyroptosis, phagocytosis")[51]. These cells primarily travel through the lymph system and the blood for metastasis, making them viable targets for biomarkers via liquid biopsy[32]. However, they are present in low concentrations in blood, thus requiring specific isolation methods to create a populated sample of these rare cells (for further diagnosis)[51]. In PCR-based assay methods for ctDNA detection, ctDNA could be identified through a "signal-to-noise ratio" to identify patients that pass a minimum threshold for ctDNA detection classification[58].

The main approach is to detect mutated genes involved with the primary tumor for specific detection and personalized therapeutic direction; otherwise, screening a larger (less specific) panel of genes which may be involved in tumorigenesis may reveal particular genetic mutations in the patient[27, 41]. Additionally, ctDNA levels detected in blood (such as "frequency of cases with detectable ctDNA (%)") can indicate localized and metastatic tumors, and also preliminary data on what organ the tumor is localized at4. In a 2014 study on ctDNA in human malignancies, it was found that ctDNA generally had higher detectable levels than CTCs, indicating the efficiency of utilizing ctDNA as a biomarker for high throughput mutation detection and analysis[3, 4](Fig. 3).

In another clinical study, 49-78% of patients with localized tumors had detectable ctDNA levels, contrasting with patients with metastatic tumors that had a 86-100% frequency of harboring detachable ctDNA levels61. Therefore, detected ctDNA in the blood could indicate metastatic or locally advanced tumorigenesis. Additionally, this study noted differences in detectable levels of ctDNA depending on cancer type, localization, and advanced vs. early stages. However, current ctDNA detection methods still reveal pitfalls in having a standardized detection threshold, as therapeutically targetable mutations in next-generation-sequencing of ctDNA had varying results[1, 35, 61].

ctDNA detection techniques include PCR, digital droplet PCR (ddPCR), next-generation sequencing, and mass spectrometry. There are limitations to ctDNA detection validity, especially with regard to streamlining purification processes, expense despite high-throughput technique implication, panel specificity to cancer type, and lack of a standardized workflow[2]. In conjunction with other sequencing and biomarker detection methods to isolate these populations, ctDNA analysis could be crucial for identifying DNA mutations leading to cancer diagnosis.

Exosomes: Exosomes are produced by all cells and are extracellular vesicles which carry lipids, metabolites, nucleic acids, and proteins in most fluids in the body. As a result, due to their guaranteed presence in all biological fluids and ability to have a multicomponent analysis, they are ideal for lipid biopsy—most notably with long term disease progression[23]. Traditional validated exosomal markers have included CD63, CD81, CD9. These are good for distinguishing high sensitivity of cancerous vs noncancerous from plasma samples with the use of CD9-CD63 in SiMoa immunoassays utilizing Single Molecule array technology assays that were developed recently from the Wei lab to

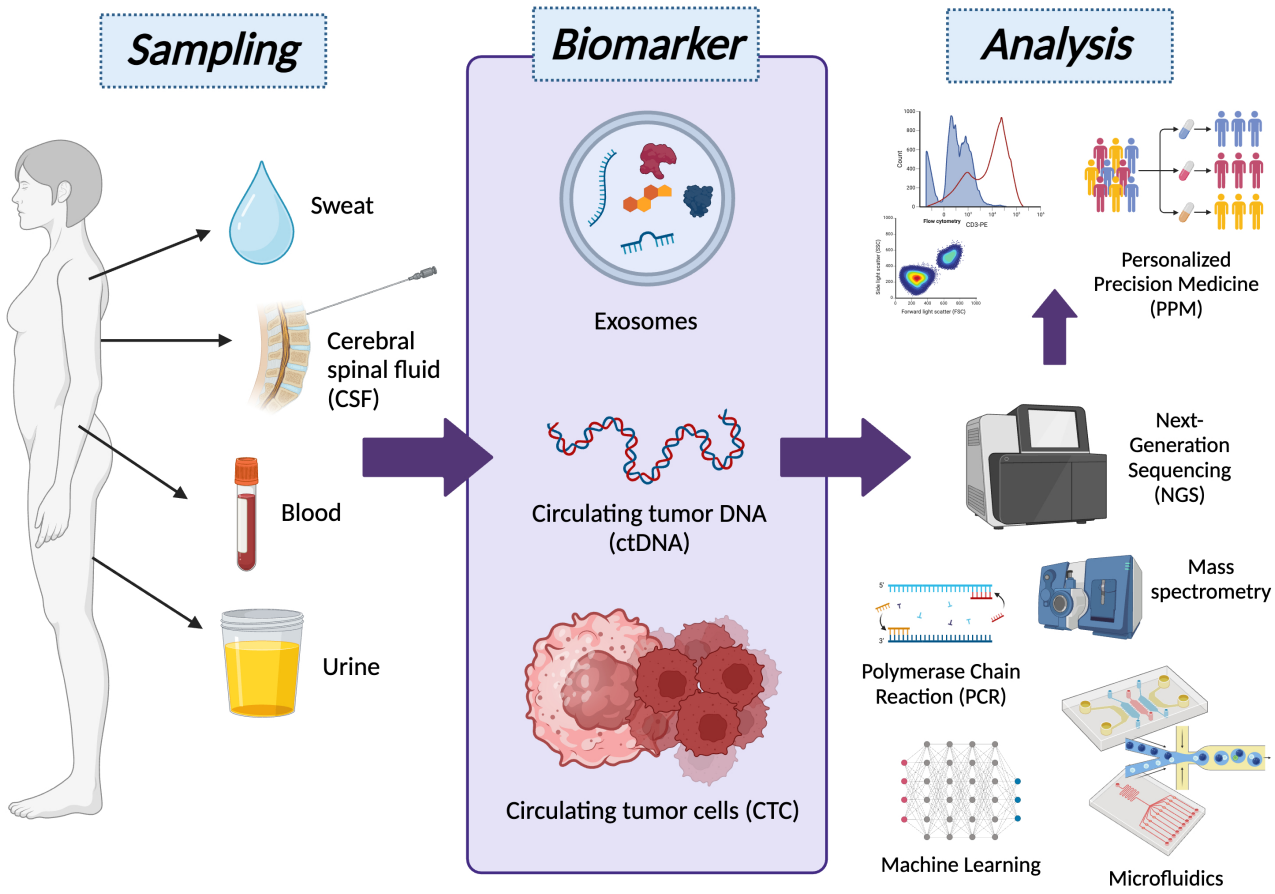

Figure 1: Overview of liquid biopsy sampling, common biomarkers, and applications. Sampling types include sweat, cerebral spinal fluid (CSF), blood, and urine, which are processed for biomarker detection and further analyzed using technology methods pictured above. Analyzed data is applied to personalized precision medicine (PPM) treatment plans for patients.

characterize exosomes[57]. CD81 is considered a housekeeping marker of exosomes and has been used, for example, in distinguishing whether a patient had prostate cancer or not based on levels of CD81 expression; higher CD81 expression is associated with prostate cancer presence[34, 55].

However there are also novel exosome markers such as the promising exosomal programmed death-ligand 1 (PD-L1) we will focus on in this review. It is a membrane-bound ligand located on the surface of various tumor cell types that binds to and suppresses the activation of programmed cell death protein-1 (PD-1) on T cells; this allows the tumor cells to evade the immune elimination52. However, PD-L1 is not only located on the surface of cells but also the surface of exosomes where it is known as exosomal PD-L152. Recent studies have shown promising implications for exosomal PD-L1 regarding detection in melanoma, head and neck cancer, glioblastoma, lung cancer, prostate cancer, breast cancer, and additional tumors[9, 41, 52, 53, 59].

Some specific advantages that exosomal PD-L1 has includes the following: it is more widely spread, more easily attached to target cells (promoting effective immune escape), analyzed at different time points predicting active disease progression, and reflects the whole body system[52]. As the methods for exosomal PD-L1 detection include ddPCR, ELISA, and flow cytometry which are all non-invasive, it means analysis for exosomal PD-L1 can be performed at multiple time points—stopping tumor heterogeneity-related problems[52]. So

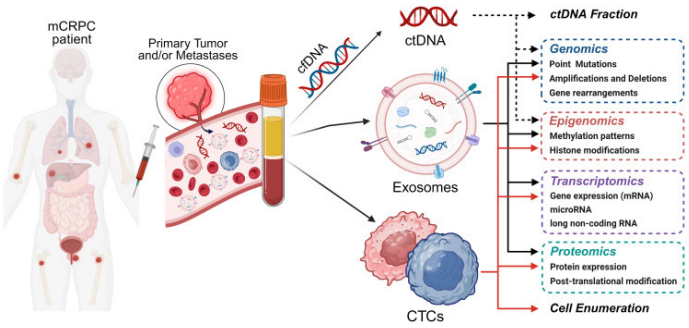

Figure 2: Types of biomarkers for liquid biopsy (mCRPC pictured) (Fig. 2, Bonfil and Al-Eyd, Oncoscience, 10, 69–80. https://doi.org/10.18632/oncoscience.592.) From blood, ctDNA, Exosomes, and CTCs, various multi-omics information is able to be extracted.

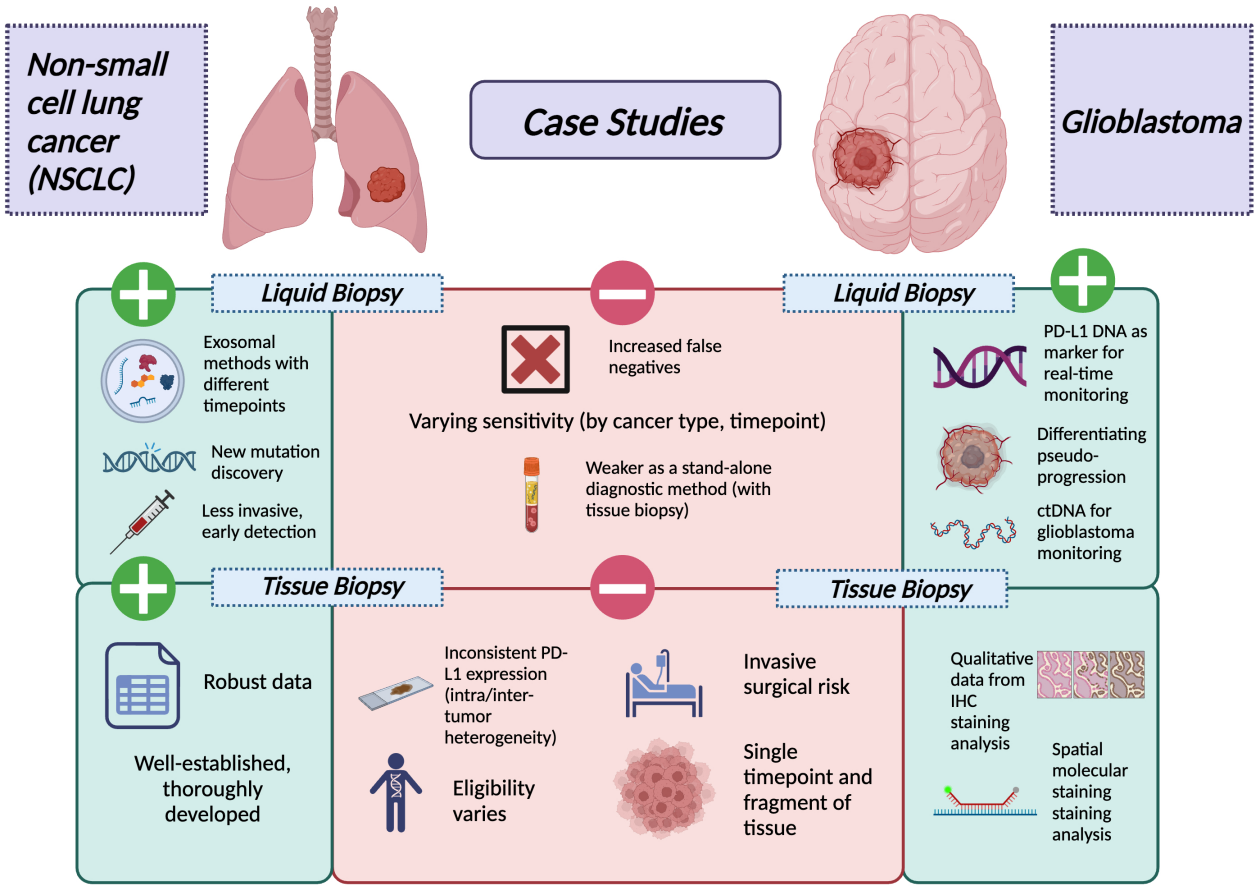

Figure 3: Case study comparisons for liquid biopsy and tissue biopsy techniques for non-small cell lung cancer (NSCLC), glioblastoma, and general cons. Liquid biopsies and tissue biopsies reveal important data points attributable to its respective sampling and analysis methods. Combined, these testing methods provide greater specificity and data for cancer diagnosis and development monitoring.

overall, exosomal PD-L1 has the optimal characteristics of both exosomes and PD-L1, since the PD-L1 expression changes to positive expression after successful treatment[52, 25]. Exosomal PD-L1 therefore is a novel diagnostic biomarker that has implications for both research and clinical areas of the oncology field[52].

However, use of exosomal PD-L1 is not without limitations such as difficulty in identifying if the observed effect is from the PD-1 blocker or the autoimmunity[52]. Further restrictions include how more large-scale clinical prospective trials must be done for conclusive evidence as thus far these results have only been from retrospective small-scale analysis[52]. Lastly, methods to manage secretion of exosomal PD-L1 by tumor cells have not been configured and future research is required to form a better mechanistic story of the way in which exosomal PD-L1 inhibits immunity[52].

CTC: Another biomarker that is notably larger are Circulating Tumor Cells, or CTCs—a mechanism by which a cancer metastasizes, or spreads, throughout the body[32]. They break off from the original cancer and travel to other parts of the body, primarily via the bloodstream[32], attempting to attach to other tissue and continue propagating.

CTCs were first observed by Ashworth Thomas Ramsden in 1869, when he noticed that there were cells in the blood of people who had passed away from cancer that were similar to the tumor. This was followed by Stephen Paget's "seed and soil" hypothesis[11]. Paget proposed that there was a seed, as in the tumor cell, and soil, a suitable, organ microenvironment[29]. However, this was not universally accepted, with many others sharing the opinion that metastasis follows a circulatory route and compromises the first encountered organ. Today, it is viewed as a combination of both, though respective extents may be unique to the tumor[29].

While metastasis has a very low success rate (<0.01%)[22], it is the leading complication in fatal cancers[22, 54] and as such, detecting CTCs and subsequent monitoring of CTC concentration are critical. However, even as the cancer progresses, CTCs are found at very low concentrations (<3 CTC/mL of blood at stage I, and >20 CTC/1mL at Stage IV)[42]. Thus, it is quite challenging to test for CTCs and ensure reliability and replicability of the results.

Knowing where CTCs circulate is among the first steps towards designing technology that can reliably detect their presence. In fact, the notion of using liquid biopsies to test for CTCs has been around for over a decade. This quickly expanded to any circulating material from a cancer[40]. Due to the difficulty of being able to reliably test for CTCs and other material, only recently is testing for CTCs becoming more feasible.

In order to test for CTCs, research has been conducted with the goal of isolating CTCs and identifying differences between them and healthy cells. CTCs from over 80% of solid cancers havebeen found to have the surface marker epithelial cell adhesion molecule (EpCAM), which peripheral red blood cells do not[22]. Another major difference is that CTCs are much larger than the cells surrounding them[22].

To take advantage of these variances, technologies like enzyme degradation, negative selection, and, more recently, functional assays and microdevices have been developed and employed to isolate and test for CTCs[22].

Technology and Applications

Liquid biopsy is applied to various analysis and diagnostic techniques for basic science research, clinical research, and personalized medicine. As technology advances, more efficient techniques are developed that provide greater specificity and quantity of crucial diagnostic or prognosis data.

PPM: Personalized Precision Medicine (PPM) is an emerging cancer treatment model. Analysis of a patients' tissues, genes, personal factors, genetic mutations, and other biological characterizations allow each treatment to be tailored for the patient[28]. For liquid biopsy detection methods that detect cancer biomarkers (such as CTCs, ctDNA, etc.), PPM utilizes the data analyzed to develop a treatment specific for a patient[28]. Nevertheless, PPM is a limited treatment option for many patients––personalized treatment is inaccessible in disadvantaged countries with fewer resources. Additionally, the genomic tools and detection methods (e.g. liquid biopsy) necessary to extract data for PPM are expensive and sometimes inaccurate or uninformative, as described in the biomarkers discussion[24]. Moreover, applications of cancer PPM through liquid biopsy diagnosis requires further research into efficacy, mortality, and improvement for cost-efficiency, given the variability of PPM and relative novelty of this treatment method[28]. Multi-omics: Multi-omics is a broad based term, encompassing various applications of genomics, transcriptomics, proteomics, spatial transcriptomics, machine learning, and more, which amalgamate to form a multidisciplinary approach to research or analysis[8]. Machine learning is at the forefront of multi-omics-based detection, especially for spatial transcriptomics of CTCs. Images can be filtered and analyzed for characteristic indicators of therapeutic efficacy, single-cell characterization, and cell fate prognosis[20]. Liquid biopsy techniques also cover transcriptomic methodologies, presenting a more targeted approach using nucleic acid markers to detect and characterize tumor types[12, 30]. Currently, liquid biopsy approaches center on utilizing numerous PCR-based and NGS techniques to analyze nucleic acid biomarkers or entire targeted genomic sequences from cancer patient samples[8]. Given that tumor biomarkers can be detected through innumerous technology strategies, it is crucial to reach a standardized protocol for liquid biopsy containment, sampling, and detection. Machine learning, among other technological approaches, can be used to mitigate and normalize biomarker detection based on cancer type and other biological or clinical factors[17, 18, 19, 33].

Microfluidics: Application of microfluidics to liquid biopsy diagnostic approaches removes the need for large and expensive equipment needed for tissue biopsy analysis; however, microfluidics approaches also special lithography and machinery for lab-on-a-chip (LOC) platforms and device making[48]. Nevertheless, the miniaturization of experiments through microfluidics greatly reduces reagent cost, time analysis, and overall cost efficiency of other diagnostic or experimental procedures. High-throughput (single-cell) analysis enables faster, more accurate detection of biomarkers relevant to cancer tumor development (e.g. CTCs, ctDNA, exosomes)[4, 5, 32, 41, 51]. Cells marked with specific biomarkers that are fluorescent-labeled[47], or have different morphologies or properties from normal blood cells, can be isolated through different microfluidic device channels[26, 31, 46, 56]. Additionally, dielectrophoresis (DEP) and field-effect transistor microfluidic chip designs have been utilized for cancer cell detection[13, 16, 38]. For nucleic acid biomarkers, other microfluidic devices that consider detection and tagging of DNA/RNA-specific biomarkers have also been developed[10, 15, 49, 60]. Schwab et al. developed the MyCTC chip to test personalized drug response of purified CTCs from patients with advanced metastasis, providing a translational approach to liquid biopsy applications in microfluidics[44].

Overall, microfluidics is a highly adaptive and personalizable technology, finding applications in cancer diagnostic approaches, personalized medicine, and research in discovering characteristics of liquid biopsy biomarkers for future translational improvements in cancer diagnostics.

Tissue Biopsy and Liquid Biopsy Comparison

NSCLC: Currently positron emission tomography/computed tomography (PET/CT) and tissue biopsy are employed for non-small cell lung cancer (NSCLC) monitoring[7]. However in NSCLC inter-tumor heterogeneity and intra-tumor heterogeneity may lead to inconsistent PD-L1 expression in biopsy tissues[45]. In addition, unfortunately not all NSCLC patients are eligible for tissue biopsy—instead it depends on the patient's clinical outcomes and tumor location[6]. Tissue biopsies also tend to have greater cost and longer turn-around times for the release of the biopsy report[6]. However, one positive aspect is that tissue biopsies tend to gather more data than liquid biopsy, and more accurately as well6. In addition through tissue biopsy a larger number of mutations were found that liquid biopsy alone did not[6]. This may partly be a result of tissue biopsies being more developed.

Meanwhile, liquid biopsy has been more recently applied to NSCLC with positive aspects such as exosomal methods that can be performed at multiple time points; they also better represent tumor heterogeneity problems with predictive biomarkers that can be detected successfully to guide treatment options[52]. In addition, more patients can get tested and more regularly, allowing for early detection[21, 52]. Liquid biopsy was also able to find a few clinically relevant mutations that did not come up in a tissue biopsy[52]. Overall it is less invasive and has quicker turn-around times.

Glioblastoma: Current diagnosis methods for Glioblastoma (GBM) include neuroimaging combined with analysis of biopsied tissue, however they both have their restrictions. Neuroimaging allows for GBM morphology to be characterized. For instance fluid-attenuated inversion recovery (FLAIR) and the more recent magnetic resonance spectroscopy are just some of the ways to characterize GBM[43]. However a drawback of neuroimaging is that it does not consistently identify GBM from other tumorous/non-tumorous diseases[43].

Tissue biopsy, has a more accurate histopathological analysis, and may highlight specific genetic and epigenetic features of GBM[43]. However it requires a surgical procedure that works for most patients, but some patients may have conditions such as deep-seated lesions or old age who would be unable to undergo surgical resection[43]. They would require image-guided stereotactic biopsy, yet that also poses surgical risk such as hemorrhage and brain swelling[43]. In addition, the small part of the tumor tissue removed for analysis, may not even be representative of the whole tumor, and therefore be an inaccurate measure[43]. Tumor tissues are also constantly evolving due to changes

in the microenvironment, so the tissue biopsies are effectively only a screenshot of the entire tumor and cannot evaluate the tumor activity in real-time as it evolves[43].

Regarding liquid biopsy, detection of CTCs was approved in 2004 for clinical use as a predictive marker of carcinomas, prostate, breast, ovarian, colorectal, lung, and other cancers[14]. Glioblastoma however did not share the epithelial marker used in the detection system, so it was unable to work then[14]. However, in 2014 rare CTCs were detected in GBM at an enhanced frequency[14]. Also, extracellular vehicles with PD-L1 DNA could provide updated monitoring of GBM progression[14]. In addition, liquid biopsy has the potential to overcome the limitations of both neuroimaging and tissue-based methods with the potential to identify early recurrence and differentiate tumor progression[14]. Other biomarkers for GBM tacking such as ctDNA have also recently been shown to be viable for both observational and interventional clinical studies[14]. Further studies will still need to be done to establish protocols and validate to suitable standards. Overall the potential for liquid biopsy as a diagnostic tool to detect mutations prior to even neurosurgical removal of the tumor has tremendous implications for reducing the tumor mass and improving outcome. Unfortunately there have been setbacks to clinical studies due to the studies being withdrawn or terminated because of the COVID-19 pandemic and lack of funding [14].

Conclusions

From our non-systematic review article inquiry, we found that liquid biopsies account for markers undetected in tissue biopsy. A liquid biopsy also grants better insight into multiple tumors or metastasis progression. It is less invasive, cost effective, and efficient. However, liquid biopsies currently maintain questionable validity. Future liquid biopsy developments may provide even more detailed comprehensive analysis, sensitivity, and accuracy.

Acknowledgements

We would like to thank Dr. Sushruta Surappa, PhD (postdoctoral scholar at Stanford's Canary Center for Cancer Early Detection) for his mentorship, guidance, and experience in novel liquid biopsy research.

References

[1] Abbosh, C., Birkbak, N. J., & Swanton, C. (2018). Early stage NSCLC — challenges to implementing ctdna-based screening and MRD detection. Nature Reviews Clinical Oncology, 15(9), 577–586. https://doi.org/10.1038/s41571-018-0058-3

[2] Adashek, J. J., Janku, F., & Kurzrock, R. (2021). Signed in blood: Circulating tumor DNA in cancer diagnosis, treatment and screening. Cancers, 13(14), 3600. https://doi.org/10.3390/cancers13143600

[3] Aggarwal, C., Thompson, J. C., Black, T. A., Katz, S. I., Fan, R., Yee, S. S., Chien, A. L., Evans, T. L., Bauml, J. M., Alley, E. W., Ciunci, C. A., Berman, A. T., Cohen, R. B., Lieberman, D. B., Majmundar, K. S., Savitch, S. L., Morrissette, J. J., Hwang, W.-T., Elenitoba-Johnson, K. S., … Carpenter, E. L. (2019). Clinical implications of plasma-based genotyping with the delivery of personalized therapy in metastatic non–small cell lung cancer. JAMA Oncology, 5(2), 173. https://doi.org/10.1001/jamaoncol.2018.4305

[4] Bettegowda, C., Sausen, M., Leary, R. J., Kinde, I., Wang, Y., Agrawal, N., Bartlett, B. R., Wang, H., Luber, B., Alani, R. M., Antonarakis, E. S., Azad, N. S., Bardelli, A., Brem, H., Cameron, J. L., Lee, C. C., Fecher, L. A., Gallia, G. L., Gibbs, P., … Diaz, L. A. (2014). Detection of circulating tumor DNA in early- and late-stage human malignancies. Science Translational Medicine, 6(224). https://doi.org/10.1126/scitranslmed.3007094

[5] Campos, C. D. M., Jackson, J. M., Witek, M. A., & Soper, S. A. (2018). Molecular profiling of liquid biopsy samples for precision medicine. The Cancer Journal, 24(2), 93–103. https://doi.org/10.1097/ppo.0000000000000311

[6] Casagrande, G. M., Silva, M. de, Reis, R. M., & Leal, L. F. (2023). Liquid biopsy for lung cancer: Up-to-date and perspectives for screening programs. International Journal of Molecular Sciences, 24(3), 2505. https://doi.org/10.3390/ijms24032505

[7] Chao, F., & Zhang, H. (2012). PET/CT in the staging of the non-small-cell lung cancer. Journal of Biomedicine and Biotechnology, 2012, 1–8. https://doi.org/10.1155/2012/783739

[8] Chen, C., Wang, J., Pan, D., Wang, X., Xu, Y., Yan, J., Wang, L., Yang, X., Yang, M., & Liu, G. (2023). Applications of multi-omics analysis in human diseases. MedComm, 4(4). https://doi.org/10.1002/mco2.315

[9] Chen, G., Huang, A. C., Zhang, W., Zhang, G., Wu, M., Xu, W., Yu, Z., Yang, J., Wang, B., Sun, H., Xia, H., Man, Q., Zhong, W., Antelo, L. F., Wu, B., Xiong, X., Liu, X., Guan, L., Li, T., … Guo, W. (2018). Exosomal PD-L1 contributes to immunosuppression and is associated with anti-PD-1 response. Nature, 560(7718), 382–386. https://doi.org/10.1038/s41586-018-0392-8

[10] Cheng, L., Sharples, R. A., Scicluna, B. J., & Hill, A. F. (2014). Exosomes provide a protective and enriched source of MIRNA for biomarker profiling compared to intracellular and cell-free blood. Journal of Extracellular Vesicles, 3(1). https://doi.org/10.3402/jev.v3.23743

[11] De Renzi, G., De Marco, G., De Meo, M., Del Rosso, E., Gazzaniga, P., & Nicolazzo, C. (2022). In vitro cultures of circulating tumor cells: A potential tool to unravel drug sensitivity. Cancer Drug Resistance. https://doi.org/10.20517/cdr.2021.121

[12] Di Sario, G., Rossella, V., Famulari, E. S., Maurizio, A., Lazarevic, D., Giannese, F., & Felici, C. (2023). Enhancing clinical potential of liquid biopsy through a multi-omic approach: A systematic review. Frontiers in Genetics, 14. https://doi.org/10.3389/fgene.2023.1152470

[13] Do, L. Q., Thuy, H. T., Bui, T. T., Dau, V. T., Nguyen, N.-V., Duc, T. C., & Jen, C.-P. (2018). Dielectrophoresis microfluidic enrichment platform with built-in capacitive sensor for Rare tumor cell detection. BioChip Journal, 12(2), 114–122. https://doi.org/10.1007/s13206-017-2204-x

[14] Eibl, R. H., & Schneemann, M. (2023). Liquid biopsy and glioblastoma. Exploration of Targeted Anti-Tumor Therapy, 4(1), 28–41. https://doi.org/10.37349/etat.2023.00121

[15] Graybill, R. M., & Bailey, R. C. (2015). Emerging biosensing approaches for microrna analysis. Analytical Chemistry, 88(1), 431–450. https://doi.org/10.1021/acs.analchem.5b04679

[16] Gu, W., & Zhao, Y. (2010). Cellular electrical impedance spectroscopy: An emerging technology of microscale biosensors. Expert Review of Medical Devices, 7(6), 767–779. https://doi.org/10.1586/erd.10.47

[17] Guo, Z., Lin, X., Hui, Y., Wang, J., Zhang, Q., & Kong, F.

(2022). Circulating tumor cell identification based on Deep Learning. Frontiers in

Oncology, 12, https://doi.org/10.3389/fonc.2022.843879

[18] He, Bingsheng, Dai, C., Lang, J., Bing, P., Tian, G., Wang, B., & Yang, J. (2020). A machine learning framework to trace tumor tissue-of-origin of 13 types of cancer based on DNA somatic mutation. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, 1866(11), 165916. https://doi.org/10.1016/j.bbadis.2020.165916

[19] He, Binsheng, Lang, J., Wang, B., Liu, X., Lu, Q., He, J., Gao, W., Bing, P., Tian, G., & Yang, J. (2020). Toome: A novel computational framework to infer cancer tissue-of-origin by integrating both gene mutation and expression. Frontiers in Bioengineering and Biotechnology, 8. https://doi.org/10.3389/fbioe.2020.00394

[20] He, Binsheng, Lu, Q., Lang, J., Yu, H., Peng, C., Bing, P., Li, S., Zhou, Q., Liang, Y., & Tian, G. (2020). A new method for CTC images recognition based on machine learning. Frontiers in Bioengineering and Biotechnology, 8. https://doi.org/10.3389/fbioe.2020.00897

[21] Ilié, M., & Hofman, P. (2016). Pros: Can tissue biopsy be replaced by liquid biopsy? Translational Lung Cancer Research, 5(4), 420–423. https://doi.org/10.21037/tlcr.2016.08.06

[22] Ju, S., Chen, C., Zhang, J., Xu, L., Zhang, X., Li, Z., Chen, Y., Zhou, J., Ji, F., & Wang, L. (2022). Detection of circulating tumor cells: Opportunities and challenges. Biomarker Research, 10(1). https://doi.org/10.1186/s40364-022-00403-2

[23] Kalluri, R., & LeBleu, V. S. (2020). The biology, function, and biomedical applications of exosomes. Science, 367(6478). https://doi.org/10.1126/science.aau6977

[24] Kasztura, M., Richard, A., Bempong, N.-E., Loncar, D., & Flahault, A. (2019). Cost-effectiveness of precision medicine: A scoping review. International Journal of Public Health, 64(9), 1261–1271. https://doi.org/10.1007/s00038-019-01298-x

[25] Kelly, R. J., Zaidi, A. H., Smith, M. A., Omstead, A. N., Kosovec, J. E., Matsui, D., Martin, S. A., DiCarlo, C., Werts, E. D., Silverman, J. F., Wang, D. H., & Jobe, B. A. (2018). The dynamic and transient immune microenvironment in locally advanced esophageal adenocarcinoma post chemoradiation. Annals of Surgery, 268(6), 992–999. https://doi.org/10.1097/sla.0000000000002410

[26] Kim, M. S., Sim, T. S., Kim, Y. J., Kim, S. S., Jeong, H., Park, J.-M., Moon, H.-S., Kim, S. I., Gurel, O., Lee, S. S., Lee, J.-G., & Park, J. C. (2012). SSA-moa: A novel CTC isolation platform using selective size amplification (SSA) and a multi-obstacle architecture (MOA) filter. Lab on a Chip, 12(16), 2874. https://doi.org/10.1039/c2lc40065k

[27] Kim, S. T., Lee, W.-S., Lanman, R. B., Mortimer, S., Zill, O. A., Kim, K.-M., Jang, K. T., Kim, S.-H., Park, S. H., Park, J. O., Park, Y. S., Lim, H. Y., Eltoukhy, H., Kang, W. K., Lee, W. Y., Kim, H.-C., Park, K., Lee, J., & Talasaz, A. (2015). Prospective blinded study of somatic mutation detection in cell-free DNA utilizing a targeted 54-gene next generation sequencing panel in metastatic solid tumor patients. Oncotarget, 6(37), 40360–40369. https://doi.org/10.18632/oncotarget.5465

[28] Krzyszczyk, P., Acevedo, A., Davidoff, E. J., Timmins, L. M., Marrero-Berrios, I., Patel, M., White, C., Lowe, C., Sherba, J. J., Hartmanshenn, C., O'Neill, K. M., Balter, M. L., Fritz, Z. R., Androulakis, I. P., Schloss, R. S., & Yarmush, M. L. (2018). The growing role of precision and personalized medicine for cancer treatment. TECHNOLOGY, 06(03n04), 79–100. https://doi.org/10.1142/s2339547818300020

[29] Langley, R. R., & Fidler, I. J. (2011). The seed and soil hypothesis revisited—the role of tumor-stroma interactions in metastasis to different organs. International Journal of Cancer, 128(11), 2527–2535. https://doi.org/10.1002/ijc.26031

[30] Li, H., Li, M., Guo, H., Lin, G., Huang, Q., & Qiu, M. (2022). Integrative analyses of circulating mrna and lncRNA expression profile in plasma of lung cancer patients. Frontiers in Oncology, 12. https://doi.org/10.3389/fonc.2022.843054

[31] Li, P., Mao, Z., Peng, Z., Zhou, L., Chen, Y., Huang, P.-H., Truica, C. I., Drabick, J. J., El-Deiry, W. S., Dao, M., Suresh, S., & Huang, T. J. (2015). Acoustic separation of circulating tumor cells. Proceedings of the National Academy of Sciences, 112(16), 4970–4975. https://doi.org/10.1073/pnas.1504484112

[32] Lin, D., Shen, L., Luo, M., Zhang, K., Li, J., Yang, Q., Zhu, F., Zhou, D., Zheng, S., Chen, Y., & Zhou, J. (2021). Circulating tumor cells: Biology and clinical significance. Signal Transduction and Targeted Therapy, 6(1). https://doi.org/10.1038/s41392-021-00817-8

[33] Liu, X., Li, L., Peng, L., Wang, B., Lang, J., Lu, Q., Zhang, X., Sun, Y., Tian, G., Zhang, H., & Zhou, L. (2020). Predicting cancer tissue-of-origin by a machine learning method using DNA somatic mutation data. Frontiers in Genetics, 11. https://doi.org/10.3389/fgene.2020.00674

[34] Logozzi, M., Angelini, D. F., Giuliani, A., Mizzoni, D., Di Raimo, R., Maggi, M., Gentilucci, A., Marzio, V., Salciccia, S., Borsellino, G., Battistini, L., Sciarra, A., & Fais, S. (2019). Increased plasmatic levels of PSA-expressing exosomes distinguish prostate cancer patients from benign prostatic hyperplasia: A prospective study. Cancers, 11(10), 1449. https://doi.org/10.3390/cancers11101449

13

[35] Mansukhani, S., Barber, L. J., Kleftogiannis, D., Moorcraft, S. Y., Davidson, M., Woolston, A., Proszek, P. Z., Griffiths, B., Fenwick, K., Herman, B., Matthews, N., O'Leary, B., Hulkki, S., Gonzalez De Castro, D., Patel, A., Wotherspoon, A., Okachi, A., Rana, I., Begum, R., … Gerlinger, M. (2018). Ultra-sensitive mutation detection and genome-wide DNA copy number reconstruction by error-corrected circulating tumor DNA sequencing. Clinical Chemistry, 64(11), 1626–1635. https://doi.org/10.1373/clinchem.2018.289629

[36] Micalizzi, D. S., Maheswaran, S., & Haber, D. A. (2017). A conduit to metastasis: Circulating tumor cell biology. Genes & Development, 31(18), 1827–1840. https://doi.org/10.1101/gad.305805.117

[37] Munari, E., Zamboni, G., Lunardi, G., Marchionni, L., Marconi, M., Sommaggio, M., Brunelli, M., Martignoni, G., Netto, G. J., Hoque, M. O., Moretta, F., Mingari, M. C., Pegoraro, M. C., Inno, A., Paiano, S., Terzi, A., Cavazza, A., Rossi, G., Mariotti, F. R., … Bogina, G. (2018). PD-L1 expression heterogeneity in non–small cell lung cancer: Defining criteria for harmonization between biopsy specimens and whole sections. Journal of Thoracic Oncology, 13(8), 1113–1120. https://doi.org/10.1016/j.jtho.2018.04.017

[38] Nguyen, N.-V., & Jen, C.-P. (2018). Impedance detection integrated with DIELECTROPHORESIS enrichment platform for lung circulating tumor cells in a microfluidic channel. Biosensors and Bioelectronics, 121, 10–18. https://doi.org/10.1016/j.bios.2018.08.059

[39] Noor, J., Chaudhry, A., Noor, R., & Batool, S. (2023). Advancements and applications of liquid biopsies in oncology: A narrative review. Cureus. https://doi.org/10.7759/cureus.42731

[40] Pantel, K. (2021). Liquid biopsy: Blood-based analyses of ctdna and ctcs. Clinical Chemistry, 67(11), 1437–1439. https://doi.org/10.1093/clinchem/hvab168

[41] Perkins, G., Yap, T. A., Pope, L., Cassidy, A. M., Dukes, J. P., Riisnaes, R., Massard, C., Cassier, P. A., Miranda, S., Clark, J., Denholm, K. A., Thway, K., Gonzalez De Castro, D., Attard, G., Molife, L. R., Kaye, S. B., Banerji, U., & de Bono, J. S. (2012). Multi-

purpose utility of circulating plasma DNA testing in patients with advanced cancers. PLoS ONE, 7(11). https://doi.org/10.1371/journal.pone.0047020

[41] Ricklefs, F. L., Alayo, Q., Krenzlin, H., Mahmoud, A. B., Speranza, M. C., Nakashima, H., Hayes, J. L., Lee, K., Balaj, L., Passaro, C., Rooj, A. K., Krasemann, S., Carter, B. S., Chen, C. C., Steed, T., Treiber, J., Rodig, S., Yang, K., Nakano, I., … Chiocca, E. A. (2018). Immune evasion mediated by PD-L1 on glioblastoma-derived extracellular vesicles. Science Advances, 4(3). https://doi.org/10.1126/sciadv.aar2766

[42] Ried, K., Eng, P., & Sali, A. (2017). Screening for circulating tumour cells allows early detection of cancer and monitoring of treatment effectiveness: An observational study. Advances in Cancer Prevention, 02(02). https://doi.org/10.4172/2472-0429.1000123

[43] Ronvaux, L., Riva, M., Coosemans, A., Herzog, M., Rommelaere, G., Donis, N., D'Hondt, L., & Douxfils, J. (2022). Liquid biopsy in glioblastoma. Cancers, 14(14), 3394. https://doi.org/10.3390/cancers14143394

[44] Schwab, F. D., Scheidmann, M. C., Ozimski, L. L., Kling, A., Armbrecht, L., Ryser, T., Krol, I., Strittmatter, K., Nguyen-Sträuli, B. D., Jacob, F., Fedier, A., Heinzelmann-Schwarz, V., Wicki, A., Dittrich, P. S., & Aceto, N. (2022). MYCTC chip: Microfluidic-based drug screen with patient-derived tumour cells from liquid biopsies. Microsystems & Nanoengineering, 8(1). https://doi.org/10.1038/s41378-022-00467-y

[45] Shen, X., Wang, Y., Jin, Y., Zheng, Q., Shen, L., Chen, Y., & Li, Y. (2021). PD-L1 expression in non-small cell lung cancer: Heterogeneity by pathologic types, tissue sampling and metastasis. Journal of Thoracic Disease, 13(7), 4360–4370. https://doi.org/10.21037/jtd-21-388

[46] Shen, Z., Wu, A., & Chen, X. (2017). Current detection technologies for circulating tumor cells. Chemical Society Reviews, 46(8), 2038–2056. https://doi.org/10.1039/c6cs00803h

[47] Shi, J., Zhang, Y., Fan, Y., Liu, Y., & Yang, M. (2024). Recent advances in droplet-based microfluidics in liquid biopsy for cancer diagnosis. Droplet, 3(1). https://doi.org/10.1002/dro2.92

[48] Sierra, J., Marrugo-Ramírez, J., Rodriguez-Trujillo, R., Mir, M., & Samitier, J. (2020). Sensor-integrated microfluidic approaches for liquid biopsies applications in early detection of cancer. Sensors, 20(5), 1317. https://doi.org/10.3390/s20051317

[49] Slouka, Z., Senapati, S., Shah, S., Lawler, R., Shi, Z., Stack, M. S., & Chang, H.-C. (2015). Integrated, DC voltage-driven nucleic acid diagnostic platform for real sample analysis: Detection of oral cancer. Talanta, 145, 35–42. https://doi.org/10.1016/j.talanta.2015.04.083

[50] Surappa, S., Multani, P., Parlatan, U., Sinawang, P. D., Kaifi, J., Akin, D., & Demirci, U. (2023). Integrated "lab-on-a-chip" microfluidic systems for isolation, enrichment, and analysis of cancer biomarkers. Lab on a Chip, 23(13), 2942–2958. https://doi.org/10.1039/d2lc01076c

[51] Sánchez-Herrero, E., Serna-Blasco, R., Robado de Lope, L., González-Rumayor, V., Romero, A., & Provencio, M. (2022). Circulating tumor DNA as a cancer biomarker: An overview of biological features and factors that may impact on ctdna analysis. Frontiers in Oncology, 12. https://doi.org/10.3389/fonc.2022.94325314

[52] Tang, Y., Zhang, P., Wang, Y., Wang, J., Su, M., Wang, Y., Zhou, L., Zhou, J., Xiong, W., Zeng, Z., Zhou, Y., Nie, S., & Liao, Q. (2020). The biogenesis, biology, and clinical significance of exosomal PD-L1 in cancer. Frontiers in Immunology, 11. https://doi.org/10.3389/fimmu.2020.00604

[53] Theodoraki, M.-N., Yerneni, S. S., Hoffmann, T. K., Gooding, W. E., & Whiteside, T. L. (2018). Clinical significance of PD-L1+ exosomes in plasma of head and neck cancer patients. Clinical Cancer Research, 24(4), 896–905. https://doi.org/10.1158/1078-0432.ccr-17-2664

[54] Visal, T. H., den Hollander, P., Cristofanilli, M., & Mani, S. A. (2022). Circulating tumour cells in the -omics era: How far are we from achieving the 'singularity'? British Journal of Cancer, 127(2), 173–184. https://doi.org/10.1038/s41416-022-01768-9

[55] Wang, X., Tian, L., Lu, J., & Ng, I. O.-L. (2022). Exosomes and cancer - diagnostic and prognostic biomarkers and therapeutic vehicle. Oncogenesis, 11(1). https://doi.org/10.1038/s41389-022-00431-5

[56] Warkiani, M. E., Khoo, B. L., Wu, L., Tay, A. K., Bhagat, A. A., Han, J., & Lim, C. T. (2015). Ultra-fast, label-free isolation of circulating tumor cells from blood using spiral microfluidics. Nature Protocols, 11(1), 134–148. https://doi.org/10.1038/nprot.2016.003

[57] Wei, P., Wu, F., Kang, B., Sun, X., Heskia, F., Pachot, A., Liang, J., & Li, D. (2020). Plasma extracellular vesicles detected by single molecule array technology as a liquid biopsy for colorectal cancer. Journal of Extracellular Vesicles, 9(1). https://doi.org/10.1080/20013078.2020.1809765

[58] Wyatt, A. W., Annala, M., Aggarwal, R., Beja, K., Feng, F., Youngren, J., Foye, A., Lloyd, P., Nykter, M., Beer, T. M., Alumkal, J. J., Thomas, G. V., Reiter, R. E., Rettig, M. B., Evans, C. P., Gao, A. C., Chi, K. N., Small, E. J., & Gleave, M. E. (2017). Concordance of circulating tumor DNA and matched metastatic tissue biopsy in prostate cancer. JNCI: Journal of the National Cancer Institute, 109(12). https://doi.org/10.1093/jnci/djx118

[59] Yang, Y., Li, C.-W., Chan, L.-C., Wei, Y., Hsu, J.-M., Xia, W., Cha, J.-H., Hou, J., Hsu, J. L., Sun, L., & Hung, M.-C. (2018). Exosomal PD-L1 harbors active defense function to suppress T cell killing of breast cancer cells and promote tumor growth. Cell Research, 28(8), 862–864. https://doi.org/10.1038/s41422-018-0060-4

[60] Zhang, P., Crow, J., Lella, D., Zhou, X., Samuel, G., Godwin, A. K., & Zeng, Y. (2018). Ultrasensitive quantification of tumor mrnas in extracellular vesicles with an integrated microfluidic digital analysis chip. Lab on a Chip, 18(24), 3790–3801. https://doi.org/10.1039/c8lc01071d

[61] Zill, O. A., Banks, K. C., Fairclough, S. R., Mortimer, S. A., Vowles, J. V., Mokhtari, R., Gandara, D. R., Mack, P. C., Odegaard, J. I., Nagy, R. J., Baca, A. M., Eltoukhy, H., Chudova, D. I., Lanman, R. B., & Talasaz, A. (2018). The landscape of actionable genomic alterations in cell-free circulating tumor DNA from 21,807 advanced cancer patients. Clinical Cancer Research, 24(15), 3528–3538. https://doi.org/10.1158/1078-0432.ccr-17-3837