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REVIEW ARTICLE

A Systematic Review of Emerging Liquid Biopsy Techniques for the Detection of Melanoma

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ABSTRACT: Melanoma cancer is in the top 10 most diagnosed cancers worldwide yearly, causing around 57,000 deaths annually. Liquid biopsy in melanoma provides a novel staging and management tool that, in conjunction with tissue biopsies, can revolutionize treatment and care. It offers sufficient information about the melanoma in the patient's body by looking at various biomarkers present in bodily fluids. This systematic review provides a recent overview of rising liquid biopsy techniques for the detection and treatment response of melanoma. A total of 30 papers were compiled from 2019-2024. Results depict that ctDNA is the prominent biomarker used in the detection and treatment monitoring of stage III and IV melanoma. NGS and PCR-based assays are prominent genetic analysis tools for mutation analysis. There is a lack of use of liquid biopsy in early-stage melanoma for detection and treatment surveillance. Liquid biopsy is the future of the management, treatment, and survival of melanoma cancer patients; therefore, this needs to be addressed in more research.

KEYWORDS: Translational Medical Sciences, Disease Detection and Diagnoses, Melanoma Cancer, Liquid Biopsy, Biomarkers.

Introduction

In individuals ages 25-39, melanoma cancer is the 3rd most diagnosed cancer in the United States.[1] In 2013, 7,990 people died of melanoma, 1.3% of all cancer deaths globally.[2] By providing adequate screening and early treatment, these deaths could have been prevented, as melanoma cancer patients' 5-year survival rate is 99% if detection can occur before the cancer spreads to the lymph nodes. Therefore, it is important to prioritize regular check-ups for any suspicious symptoms because early detection might improve prognosis even more. As with most cancers, the earlier they are diagnosed, the better the outcome.

Between 2015 and 2019, melanoma mortality decreased by 4% every year because of the introduction of new immunotherapy drugs on the market.[3] This shows that providing treatment can help significantly improve the survival rate, but it can only be done if the patient is diagnosed early. If the disease is detected early and monitored during treatment using advanced methods such as liquid biopsy to identify and monitor a specific set of mutations that occur in DNA in the blood, the patient's mortality rate could decrease.

Liquid biopsy studies biomarkers in body fluids and fluid connective tissues such as blood, urine, tears, and CSF rather than regular tissues. Biomarkers are molecular and genetic indicators of normal or abnormal processes in a person's body, in this case, from cancerous and normal cells. Some biomarkers used in oncology are circulating tumor DNA (ctDNA), circulating cell-free DNA (cfDNA), circulating tumor cells (CTC), and tumor mutational burden (TMB).[4] Liquid biopsy is an essential tool that can be used to gain information on tumor possibility and mutations in your genetic code at a molecular level that imaging through a PET scan can't show us.

There are multiple reasons why blood tests are done on known cancer patients. Firstly, because of guided therapy. In guide therapy, if whole cells can be recovered, then they can be sequenced for specific mutations. Additionally, if ctDNA, cfDNA, or CTC levels go up, then doctors can know that the patient has relapsed. Commonly, when cfDNA levels it might trigger a full-body PET scan to look for recurrence.

One of the many benefits of liquid biopsy is personalized treatment. Personalized medicine is a new frontier extremely beneficial to cancer patients because each patient is treated according to their genetic profile. This can potentially improve the management of patients, minimize side effects, and increase survival rates. The benefit of using ctDNA or cfDNA from liquid biopsies is that the treatment will be able to combat unique problems for each specific patient, instead of having a generalized approach to various problems. This can solve not just the overarching problem, but also the small ones, in a patient, ensuring they live long, healthy lives. Another benefit, as mentioned above, is that it is noninvasive. Normal biopsies, while they are tiny and not very invasive, still require surgery to collect tissue for analysis and therefore put the patient at risk for bleeding, infections, and scarring. But with a liquid biopsy, similar critical information can be accessed completely noninvasively.

However, there are drawbacks and challenges. One challenge is that the concentration of tumor DNA available in the blood is not high enough. Cells, during apoptosis, shed only a little bit of their DNA into the blood because it is packaged into bound vesicles called apoptotic bodies. The rest is degraded by phagocytic cells. Therefore, amplification is required after extraction, usually based on a PCR test, to increase the targeted sequence amount so that mutations can be detected. With liquid biopsies, false negatives are also common in clinical settings

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because of the low amount of tumor DNA that is not detected by the biopsy. Thus, the doctors there would probably have to conduct a tissue biopsy, defeating the noninvasive benefit of liquid biopsies. To find rare mutations in ctDNA or cfDNA, detected in liquid biopsy, Next Generation Sequencing (NGS) is conducted. However, it is quite expensive. It was found that for Targeted Panel Testing, sequencing cancer-specific genes costs around 2100 dollars. For whole genome and comprehensive genome profiling, it costs even more, around 4950 and 3420 dollars. Thus, not all clinics worldwide can afford to conduct NGS for each melanoma cancer patient. Before the implication of any treatment based on data from liquid biopsy, there must be a thorough analysis of the results with proper health authorities and prominent scientists, which has not yet been implemented and will take time, too.

While liquid biopsy isn't an emerging technique for melanoma, there are others that are being studied more recently for detection. For example, by using artificial intelligence to analyze pictures of different skin lesions, doctors can evaluate their diagnosis with another source. Additionally, total body photography allows doctors to diagnose high-risk patients who most likely have a family history of melanoma. Dermoscopy, a process that uses a dermoscope to magnify sub-surface skin structures, is also becoming more sensitive and efficient to use to identify malignant melanoma in patients. Not necessarily a new technique, but multimodal approaches have become more common, so evidence can be corroborated by multiple sources. By using these new imaging and photography in conjunction with liquid biopsy, NGS, and immunotherapies for melanoma cancer detection and treatment, patients' survival can be key to happen.

Methods

Research on melanoma and liquid biopsy has been a prominent topic in the scientific community in the last 30 years. By targeting specific genes and using a broad range of technological tools for collecting data, scientists are able to understand how to prevent, locate, and treat melanoma cancer. This paper analyzes a small portion of the most recent research done.

A list of 30 papers on melanoma, liquid biopsy, biomarkers, and new treatments in the field, ranging from 2019 to 2023, was compiled for a literature search. Some key terms for search include "melanoma and liquid biopsy" and "melanoma and ctDNA." This information was summarized into a table with headings of methods, target genes, biomarkers, stage of cancer, and more. I've also stated the limitations and the future of liquid biopsy in melanoma cancer in the discussions. The papers that were selected adhered to specific criteria: non-systematic reviews and had to be only in the English language.

Results

Table 1: Compilation of scientific papers. Findings illustrate that ctDNA is the most prominent biomarker used. NGS and PCR are the most common tools, and analysis of PD-L1 is not common.

Author (Year)	Method	Target Gene	Research Question/ Topic	Biomarker	Tumor Stage	Sample Size	Preanalytical factors
Yin He, Xiaosheng Wang (2023)	Multiomics	-	Identifying biomarkers associated with immunotherapy response in melanoma by multi-omics analysis	TMB, PD-L1 expression	-	472	-
Gabriel Velez (2021)	Using vitreous biopsies for identifying biomarkers for uveal melanoma by examining independent cohort of patients	-	Can vitreous biopsy find biomarkers associated with uveal melanoma?	SCFR, HGF, and HGFR (Proteins)	3 and 4	8	-
Deborah H Im (2022)	Illumina sequencing and targeted sequencing	BAP1 and GNAQ	Does aqueous humor of uveal melanoma have sufficient ctDNA to perform genetic analysis?	ctDNA	1	37	-
John J. Park (2021)	ddPCR (digital droplet PCR) and NGS	TP53, GNAQ	Can baseline ctDNA in metastatic UM strongly correlate with baseline LDH level and disease volume?	ctDNA	Advanced stage	17	EDTA
Russell J. Diefenbach (2022)	NGS panels	TERT	Can a custom NGS panel detect mutations in the TERT promoter region in ctDNA?	ctDNA	3 and 4	21	EDTA
Claudia Sabato (2022)	RT-qPCR and PCR	-	Can a blood test based on microRNA non-invasively detect melanoma?	microRNA	3 and 4	19	EDTA
Carlos Alberto Aya-Bonilla (2020)	RT-PCR and ddPCR	19 different genes	Can multimarker derived CTC scores be used for prognostic and treatment response in metastatic melanoma?	CTC	-	43	-
Ekaterina Galanzha (2019)	In vivo blood test	-	Can a noninvasive in vivo blood test detect CTC in melanoma bloodstream by using our PAFC Cytophone?	CTC	3 and 4	18	-
Rongzhi Huang (2020)	Targeted panel of genes	Different genes	Can the immune-related genes prognosis biomarker be an effective potential prognostic classifier in the immunotherapies and surveillance of melanoma?	CMTM6	-	905	-
Sandra Fitzgerald (2023)	NGS and ddPCR	BRAF and NRAS	Dynamic ctDNA Mutational Complexity in Patients with Melanoma Receiving Immunotherapy	ctDNA	3 and 4	29	EDTA
Jenny H. Lee (2019)	Cox regression analyses and ddPCR	BRAF, NRAS, KIT	Can ctDNA that is detected before completing surgical resection in patients with AJCC stage IIIB/C/D (high-risk stage III)	ctDNA	3	174	EDTA

			with a BRAF, NRAS or KIT mutant melanoma show that its is an independent predictor of worse MSS in patients receiving no systemic adjuvant therapy?				
Elin S. Gray (2015)	ddPCR	BRAF and NRAS	Can we use ctDNA to monitor treatment response in metastatic melanoma?	ctDNA	M1a or b	48	EDTA
Russell J. Diefenbach (2020)	NGS	30 different genes	Can stage 3 or stage 4 melanoma be monitored by NGS from ctDNA analysis?	ctDNA	3 and 4	91	
Jesper Geert Pedersen (2020)	ddPCR	BRAF, NRAS, TERT	Can levels of ctDNA, MCP1, and TNFα predict disease progression in metastatic melanoma patients with checkpoint inhibitors?	ctDNA	3 and 4	16	EDTA
Claudia H. D. Le Guin (2021)	Illumina sequencing	GNAQ/ GNA11	Can detection of cfDNA in plasma provide a diagnostic lead time diagnosis of metastases or tumor recurrence in uveal melanoma?	cfDNA	-	151	-
Akira Kaneko (2021)	ddPCR and Capp Sequencing	BRAF	Can liquid biopsy CAPP sequencing and ddPCR detect tumor presence and mutations?	cfDNA	Early stage	15	-
Zeynep Eroglu MD (2023)	multiplex PCR	-	Predictive and prognostic value of a personalized, tumor-informed ctDNA assay for the detection of molecular residual disease (MRD)	ctDNA	3 and 4	69	-
Jan Braune (2020)	ddPCR	BRAF and NRAS	Can early changes in ctDNA predict responses to treatment and ctDNA for detecting tumor burden for melanoma	ctDNA	3 and 4	62	EDTA
Andrea Forschner (2020)	Illumina sequencing and ddPCR	BRAF and MEK	Can BRAF V600 mutant ctDNA can be used to reliably determine progressive disease under targeted therapy and whether patients' prognoses are different if ctDNA is detectable before initiating targeted therapy?	ctDNA	Advanced	19	EDTA
Marina Berger (2021)	NGS	BRAF and MEK	Can NGS gene panels be used for treatment monitoring in melanoma cancer through ctDNA analysis?	ctDNA	3 and 4	31	Strek
Selena Y Lin (2020)	qRT PCR	BRAF	Can blood molecular profiling of circulating tumor cells (CTCs) enable monitoring of patients with metastatic melanoma	CTC	3 and 4	75	Strek
			during checkpoint inhibitor immunotherapy (CII) + in combination with targeted therapies?				
Anthony Lucci (2023)	CellSearch	-	investigated how frequently and how early circulating tumor cells (CTCs) were identified prior to the surveillance imaging detection of melanoma progression	CTC	3	325	-
Ioana Gencia (2020)	Real time PCR	-	Compare miRNA expression between primary melanomas from different sites	microRNA	3 and 4	32	-
John J. Park (2021)	NGS and ddPCR	GNAQ, GNA11 and CYSTLR2	Can circulating tumor DNA reflect Uveal Melanoma responses to protein Kinase C inhibition?	ctDNA	-	17	EDTA
Pawel Sobczuk (2022)	qPCR	BRAF	Can we evaluate the clinical utility of plasma circulating tumor DNA analysis for BRAF mutation?	ctDNA	3 and 4	46	-
Russel J. Diefenback (2022)	NGS	TERT, BRAF. NRAS	Detection of melanoma mutations using circulating tumor DNA (ctDNA) is a potential alternative to using genomic DNA from invasive tissue biopsies.	ctDNA	3 and 4	21	EDTA
Lydia Warburton (2020)	ddPCR and NGS	BRAF	Can ctDNA be a reliable biomarker for disease progression after treatment was stopped?	ctDNA	Advanced	70	EDTA
Joesph W. Po (2019)	Flow cytometry	PD-L1	Can a reliable melanoma circulating tumor cell (CTC) detection method evaluate PDL1 on CTC's?	CTC	4	14	-
Carolyn Hall (2018)	Cox regression analyses	-	Determine if circulating tumor cells (CTCs) are associated with shortened (180-day) progression-free survival (PFS) after a baseline CTC assessment in stage IV melanoma patients.	CTC	4	93	-

Table 2: FDA-approved single and combination drug treatments for melanoma cancer. Results depict combination drugs to be more utilized currently.

Drug/treatment	Description	Single or combination	Month and year approved by the FDA
Talimogene laherparepvec/ IMLYGIC	Genetically modified herpes virus to create immunoprotein called GM-CSF	Single	October, 2015
Ipilimumab/YERVOY	Supports activation and proliferation of T-cells strengthening the immune system	Single	March, 2011
Nivolumab and retalimab- rmb/ OPUDUALAG	Uses blocking antibodies against PD1 and a lymphocyte activating gene (LAG-3) blocking antibody	Combination	March, 2022

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For melanoma, as found in this review, ctDNA is the most common biomarker used. 16/30 or 53.3% of the results depicted that ctDNA was the biomarker used for detection in their studies, showing that currently it is the most prominent. In earlier reports, the expression of PD-L1 has been more prominent, but that is not the case now: the majority of the studies focused on BRAF and NRAS genes. The results also show that ctDNA is more sensitive to III and IV late-stage cancers, as shown in 81.3% of the 16 studies. (Table 1) However, in the early detection of melanoma, as shown in the studies gathered, the sensitivity is very low for stage I or II ctDNA (3 out of 16). This is because there is less presence of cancerous cells; therefore, fewer cells are releasing DNA. You need a large amount of ctDNA in the blood for a liquid biopsy to have a high sensitivity. Additional work must be done to be able to use liquid biopsy to aid the genetic profiling of patients with earlier stages of melanoma, so that more personalized treatments can be made and given to them. It was also found that the most common genetic analysis technique used was next-generation sequencing (NGS) and droplet digital polymerase chain reaction (ddPCR) in the studies collected (Table 1).

One way that scientists and doctors are using the immune system is checkpoint inhibition. Checkpoint inhibition is the blockage of proteins that inhibit the immune system from attacking cancerous cells in the body. It is a type of immunotherapy, a new treatment that focuses on using the immune system to stop the spread of cancer cells. Specifically, in terms of melanoma, the inhibition of the immune checkpoint inhibitor PD-1 is a key treatment that can be done by two specific drugs, pembrolizumab and nivolumab, on the market. Nivolumab is depicted in Table 2 as one of 2 in the up-and-coming combination therapy.

FDA-Approved Drug Treatments:

As shown, in previous years, the use of a single drug for melanoma treatment was common. 50% of the therapies presented show that it is a combination therapy, and they are all in the last 5-7 years. On the other hand, single-drug treatment was more common in the last 10-15 years. Therefore, combination therapies have become more prevalent. This is because of the need to combat multiple aspects of melanoma tumors in patients' treatment. These various aspects could include increasing immune system strength, targeting and blocking specific genes, and creating proteins or antibodies. In relation to liquid biopsy, the use of combination therapies allows for targeting multiple cells, proteins, or kinases that can be recognized through sequencing after liquid biopsy. Liquid biopsy can monitor this treatment as well, and adjustments to delivery, drugs, or amount can be made accordingly.

Preanalytical Factors:

Preanalytical factors were also considered for bias in this systematic review. The amount of plasma in which tumor DNA was extracted, a range between 2-40 mL, affected the study's sensitivity. The type of tube they used to store the plasma, Strek or EDTA, also introduced bias. Additionally, the type of DNA extraction done, the different protocols conducted during this process, and how they quantified their data are preanalytical factors that introduce bias in their study.

■ Discussion

The application of invasive tissue biopsy in clinical settings is a prognostic tool that, with initial tissue biopsy diagnosis to detect the depth of melanoma, can become a revolutionary tool for monitoring and treating patients. By understanding the depth of tissue biopsy, the amount of radiation to treat the patient can be determined, and liquid biopsy can be used to check for relapses.

Firstly, liquid biopsy must be more sensitive to late-stage melanoma because of the increased amount of ctDNA in the fluids. Because 83% of the ctDNA studies were utilizing stage 3 and 4 melanomas, this shows that to procure enough DNA for sequencing and analysis, they needed fluid in which there was enough DNA for a liquid biopsy to detect. Connecting back to the introduction, this demonstrates how liquid biopsy can be an essential tool for monitoring treatment progress in late-stage cancers, as ctDNA levels are high enough for analysis. This shows the advantage of using liquid biopsies rather than using tissue biopsies to check progress, which will take time, money, and risk.

Secondly, the increased prevalence of BRAF, MEK, and NRAS gene sequencing over combating the protein PDL1 shows the scientific community heading toward combating melanoma at a genetic level. 14 studies explicitly stated using those genes in their process, while only 1 explicitly stated combating PDL1. A key part of personalized medicine, as stated in the introduction, is creating treatments according to the patient's own DNA. Therefore, more research is being done toward installing personalized medicine in melanoma detection and treatment, as sequencing genes is the first step.

While these studies are thorough on liquid biopsy, biomarkers, and combination therapies, there are some limitations. These studies lacked the use of biomarkers in earlier stages of melanoma. This is because their low concentration in the plasma extracted does not enable them to be used. Therefore, there should be an increased focus on early diagnosis in future studies that use liquid biopsy as the primary diagnostic tool. Liquid biopsy is the future of the detection of melanoma cancer.

■ Conclusion

The main results of this study show that the most common biomarker used for diagnosis and prognosis is ctDNA, which is extracted by the liquid biopsy technique for stage III or IV melanoma patients. In addition, the implementation of FDA-approved combination drug treatments in melanoma over singular treatments has risen in recent years. In conclusion, liquid biopsy is the future of melanoma cancer.

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