The Use of Circulating Tumor DNA in Cancers of Unknown Primary: A Review of the Literature

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Abstract

Cancers of Unknown Primary (CUP) are not an uncommon clinical entity with high mortality rates. The application of platinum-based chemotherapy is still recommended as the Standard of Care (SOC) for patients with unfavorable CUP although with poor results. Attempts at identifying the primary tumor location with molecular assays have failed to show clinical benefit, while investigations of actionable targetable mutations are hampered by the significant molecular heterogeneity of CUP. Liquid biopsies, due to their inherent advantages such as being minimally invasive and allowing for the identification of temporal and spatial tumor heterogeneity, may provide an appealing alternative to elucidate the molecular background of CUP and tailor timely and individualized targeted and specific treatments. Although data are limited regarding the possible applications of circulating tumor DNA (ct-DNA) in CUP, they warrant further investigation.

Keywords: Cancer of unknown primary • Ct-DNA • Liquid biopsy

Introduction

Cancers of Unknown Primary (CUP) account for 2%-5% of all invasive cancers [1]. Although the incidence is not very high, their mortality rate is. The majority of patients are diagnosed with unfavorable CUP and undergo cytotoxic chemotherapy. According to available data, they do not seem to benefit from strategies aiming at the molecular identification of a primary location or actionable mutations using tissue DNA- or RNA- based techniques [2]. To date, molecular cancer classifiers as well as comprehensive gene expression profiling have both been investigated [2-4]. The former are used to identify the tissue of origin and guide site-specific therapy, whereas the latter are applied to identify actionable, druggable mutations among a broad group of genes [5-7]. In light of these efforts, molecular testing has gradually been incorporated into CUP management while pending data from ongoing clinical trials [2].

Liquid biopsies may overcome the inherent drawbacks associated with tissue biopsies. Circulating Tumor DNA (ct-DNA) has been extensively investigated in recent years, potentially providing an attractive alternative to tissue for the detection of actionable mutations and tumor molecular profiling. It is emerging as a promising biomarker due to its non-interventional nature and its availability for temporal and spatial tumor investigation with many possible clinical applications [8]. Released into the blood by cancer cells due to apoptosis, necrosis or secretion, ct-DNA has entered clinical practice in specific circumstances and clinical scenarios [8-10]. At the same time, current platforms with excellent sensitivity are available for massively parallel sequencing, even with the use of minute levels of ct-DNA in blood circulation [11].

Literature Review

Ct-DNA application in CUP

Data regarding the use of ct-DNA-based liquid biopsies in patients with CUP are scarce. A review of the literature was conducted, and a total of nine articles were found. The search strategy applied included all related terms for CUP and ct-DNA (Tables 1). A brief summary of the available data is presented in table 1 [12-20].

Table 1. Application of ct-DNA in CUP.

Publication	Year	No of Patients	No of Genes in Panel	Authors Conclusions
Kato S. *et al.* [12]	2021	1931	73-74	90% of patients had ≥ 1 cfDNA alteration, high degrees of matching to therapy correlates with better outcomes
Kato S. *et al.* [13]	2017	442	54-70	80% of patients exhibited ctDNA alterations; Distinct genomic profiles were observed in 87.9% of CUP cases with 99.7% exhibiting potentially targetable alterations
Yu B. *et al.* [14]	2022	1	Not reported	Identification of an ERBB2 Copy Number Variation (CNV), amplification, and anti-HER 2 therapy application successfully
Cox R. E. *et al.* [15]	2022	1	55	Ct-DNA testing revealed a Deficient Mismatch Repair (dMMR) tumor and immunotherapy was successfully applied
Komura A. *et al.* [16]	2023	1	>300	Identified five likely pathogenic gene variants, including *NF1* and PTEN
Laprovitera N. *et al.*	2021	2	92	The 92-gene custom panel identified 16 non-

[17]				synonymous somatic alterations in ccf-DNA
Kolbinger F. *et al.* [18]	2023	23	151	A total of 22 somatic mutations in evDNA and/or cfDNA in 11/23 patients were identified, 14 classified as Tier 1
Conway A.M. *et al.* [19]	2024	41	641	Cf-DNA mutation and methylation profiling predict tumor types in 32 out of 41 cases. A total of 345 mutations were identified across 33 patients, with 82.5% of the patients having at least one mutation
De Wilde J. *et al.* [20]	2024	13	73	The cf-DNA fraction ranged from 81% to 99%, with an average of 90%. In 10 out of 13 samples, the origin of the ct-DNA was correctly predicted

In the largest published series, researchers focused on molecular profiling of approximately 1900 patients with CUP diagnosis using a Cell-Free DNA (cf-DNA) NGS panel covering 73 genes-74 genes. In ninety percent of the patients, a genomic alteration in cf-DNA was discovered. Sequencing results were then correlated with genomically matched targeted therapies, but no improvement was demonstrated in both overall (OS) and Progression Free Survival (PFS). However, higher matching scores were significantly associated with better PFS [12].

Another study performing genomic analysis in patients with CUP enrolled 442 patients. By analyzing 54 genes-70 genes on ct-DNA, the investigators detected at least one mutation in 80% of patients, with TP53 being the most commonly mutated gene. Mutations involving the MAPK pathway and PIK3CA were also documented in 31% and 18% respectively. Ct-DNA was also used as biomarker for disease monitoring in one patient of the study. Among the tested patients, no common molecular signature was found across all cases. However, the majority harbored a potentially targetable alteration, highlighting the molecular diversity of CUP and the need for personalized management in this disease [13].

Three case reports also highlight the role of ct-DNA profiling in identifying actionable mutation and microsatellite instability, respectively, which further directed patient therapy with remarkable clinical benefit [14-16].

Laprovietra et al. have also reported the application of ctDNA in genetic testing and longitudinal monitoring of two CUP patients [17]. Somatic mutations in 23 patients with CUP were investigated using ct-DNA by Kolbonger *at al.*, revealing gene alterations in 11 out of 23 patients, some which were classified as Tier I druggable somatic variants, emphasizing the significance of liquid biopsies for possible primary tumor-independent basket and umbrella trial inclusion [18].

In Conway's study, there is an application of machine learning classifier known as CUPid in order to accurate predict the origin of CUP. This machine uses the cf-DNA methylation patterns. In the 40 out of 41 patients where cfDNA mutation profiling was successful, 345 mutations were identified across 33 patients, with 82.5% of patients having at least one mutation. In addition, the median number of mutations per patient was 5, ranging from 0 to 77, and the median Variant Allele Frequency (VAF) was 10.4%, with a range of 0 to 61.3%. Cf-DNA mutation and methylation profiling predict tumor types in 32 out of 41 cases. A total of 345 mutations were identified across 33 patients, with 82.5% of the patients having at least one mutation. The cfDNA fraction ranged from 81% to 99%, with an average of 90%. In 10 out of 13 samples, the origin of the ctDNA was correctly predicted. The most significant finding is that all 32 patients were identified as having the potential to benefit from significantly different, tumor-specific treatment strategies compared to the Standard-of-Care (SOC) chemotherapy for CUP [19].

Moreover, Wilde's study employed Cell-Free Reduced Representation Bisulfite Sequencing (cfRRBS), a technique that identifies the methylation profile from minimal amounts of highly fragmented DNA. This method was used for CUP diagnosis on Formalin-Fixed Paraffin-Embedded (FFPE) tissue and liquid biopsies. The study demonstrated that cf-DNA methylation profiling can be superior to cytology in ascites and pleural effusions, provided that the samples are of high quality with cf-DNA percentages greater than 40%. Classification is not feasible if the predicted tumor percentage is less than 7% in cf-DNA samples. When the prediction ratio is 0.5 or higher, the accuracy of the classifier reaches 100% in cfDNA samples [20].

Currently, the CUPISCO trial aims to compare the safety and efficacy of molecularly targeted therapy or immunotherapy versus platinum-based therapy in patients with unfavorable CUP based on genomic profiling. The trial found that site-specific therapy led to better PFS and OS compared to the generally applied platinum-based chemotherapy. Specifically, OS was 28.2 months for site-specific therapy versus 19.0 months for platinum-based chemotherapy (HR 0.74; 95% CI 0.52–1.06; p=0.098). Although survival was still poor, these results highlight the need for further investigations [21,22].

Is there room for ct-DNA application in CUP? Implications and limitations: To improve outcomes for CUP patients, it seems imperative to expand the diagnostic workup, as current diagnostic algorithms often fail to identify the primary location or actionable therapeutic targets in most cases. Liquid biopsies, which are now extensively studied and already implemented in clinical practice, offer a potential alternative to tumor biopsies, overcoming many inherent obstacles in cases of CUP [23]. Given the previously quoted data, there appears to be a window of opportunity for implementing liquid biopsies in CUP cases, albeit with some limitations.

Although patients with CUP present with aggressive metastatic disease, tissue acquisition is not always possible. Technical issues related to accessing metastatic lesion sites can obstruct sample collection. Even when tissue is successfully acquired, the specimen may not always be adequate, as extensive Immuno Histo Chemistry (IHC) often needs to be performed, sometimes with inconclusive results. Additionally, a tissue sample may represent only the site of acquisition, failing to capture the tumor's heterogeneous molecular portfolio. CUP is known for its diversity, and available data have failed to establish a distinct molecular signature for CUP, although specific molecular events (such as TP53 mutations) are common among CUP patients [2,24]. The application of ctDNA-based liquid biopsies may offer a solution to these difficulties in the diagnostic algorithm for patients with CUP.

The resistance to and subsequent failure of chemotherapy in patients with unfavorable CUP have historically resulted in persistently low overall survival rates. ctDNA could serve as a potential biomarker for assessing patients' responses to administered therapy and detecting early disease progression, thereby allowing for timely therapeutic interventions. Additionally, the application of liquid biopsies may be useful in identifying preexisting or new actionable mutations that emerge after chemotherapy, as serial monitoring can be employed throughout the course of the disease [25].

Hence, molecular profiling using ct-DNA can be an attractive alternative to tissue investigation for CUP. It allows for the molecular signature of the tumor to be obtained at different time points, regardless of tissue adequacy, patient performance status, or the therapy applied. Additionally, since a wide panel of genes can be tested using ct-DNA, there is potential for identifying both the otherwise occult primary tumor and possible druggable mutations [24].

On the other hand, the use of ct-DNA in CUP presents several challenges. First and foremost, the concordance between tissue and ct-DNA profiling in CUP patients has not been studied, unlike in other solid neoplasms

where highly matching results have been demonstrated [26]. Furthermore, even when actionable mutations are identified through ct-DNA profiling, clinical trial results for the application of targeted therapy in CUP have been inconclusive. The superiority of site-specific therapy over SOC chemotherapy has not yet been demonstrated, and the overall complex and diverse mutational landscape of CUP further impedes the regular application of molecular targeted therapies [2].

Where do we stand?: Despite CUP representing a promising and attractive field for ct-DNA liquid biopsy applications, the current state of the art is far from satisfactory. There are only a few studies reported in the literature, most of which are case reports or small series. The paucity of available studies affects both the understanding of the intrinsic mechanisms of CUP and the development of targeted treatments for this complex disease. Medical ct-DNA research in CUP, despite its promising potential, remains remarkably scarce and appears to be lagging behind. Currently, only two large studies by Kato S. and collaborators are leading the way, offering a glimmer of hope in a field characterized by scientific paucity [12,13].

Conclusion

In the era of rapidly evolving molecular oncology, patients with unfavorable CUP still face a poor prognosis. The inability to identify the primary tumor leads to the application of conventional chemotherapy, which often yields poor results. However, advancements in exploring the tumor's mutational landscape have been made with the use of highly sensitive NGS techniques. Concurrently, minimally invasive liquid biopsies based on ct-DNA have emerged as promising biomarkers in various solid tumors, offering a range of potential clinical applications. Despite the promise of ct-DNA research and clinical applications for CUP, data remain scarce. Large prospective clinical trials investigating the role of molecular profiling and targeted therapy are underway, and their results are eagerly awaited to facilitate individualized therapy for CUP patients and address the historical benchmarks of unfavorable prognosis. Significant research efforts are still needed in the CUP setting to fully realize the potential of ctDNA applications and keep pace with advancements in the field.

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References

1. Levi, F., et al. "Epidemiology of unknown primary tumours." *Eur J Cancer.* 38.13 (2002): 1810-1812.
2. Krämer, A., et al. "Cancer of unknown primary: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up." *Ann Oncol.* 34.3 (2023): 228-246.
3. Rassy, E., and Nicholas P. "The currently declining incidence of cancer of unknown primary." *Cancer Epidemiol.* 61 (2019): 139-141.
4. Bugat, R., et al. "Summary of the standards, options and recommendations for the management of patients with carcinoma of unknown primary site (2002)." *Br J Cancer.* 89.1 (2003): 59-66.
5. Fizazi, K., et al. "A phase III trial of empiric chemotherapy with cisplatin and gemcitabine or systemic treatment tailored by molecular gene expression analysis in patients with carcinomas of an unknown primary (CUP) site (GEFCAPI 04)." *Ann Oncol.* 30 (2019): 851.
6. Hayashi, H., et al. "Randomized phase II trial comparing site-specific treatment based on gene expression profiling with carboplatin and paclitaxel for patients with cancer of unknown primary site." *J Clin Oncol.* 37.7 (2019): 570-579.
7. Clynick, B., et al. "Genetic characterisation of molecular targets in carcinoma of unknown primary." *J Transl Med.* 16 (2018): 1-10.
8. Bettegowda, C., et al. "Detection of circulating tumor DNA in early-and late-stage human malignancies." *Sci Transl Med.* 6.224 (2014): 224-226.
9. Tivey, A., et al. "Circulating tumour DNA—looking beyond the blood." *Nat Rev Clin Oncol.* 19.9 (2022): 600-612.
10. Pascual, J., et al. "ESMO recommendations on the use of circulating tumour DNA assays for patients with cancer: a report from the ESMO Precision Medicine Working Group." *Ann Oncol.* 33.8 (2022): 750-768.
11. Kastrisiou, M., et al. "Development and Validation of a Targeted 'Liquid'NGS Panel for Treatment Customization in Patients with Metastatic Colorectal Cancer." *Diagnostics.* 11.12 (2021): 2375.
12. Kato, S., et al. "Therapeutic actionability of circulating cell-free DNA alterations in carcinoma of unknown primary." *JCO Precis Oncol.* 5 (2021): 1687-1698.
13. Kato, S., et al. "Utility of genomic analysis in circulating tumor DNA from patients with carcinoma of unknown primary." *Cancer Res.* 77.16 (2017): 4238-4246.
14. Yu, B., et al. "Case report: molecular profiling assists in the diagnosis and treatment of cancer of unknown primary." *Front Oncol.* 12 (2022): 723140.
15. Cox, R.E., and Sakti C., "A Carcinoma of Unknown Primary (CUP) Patient Treated Successfully With Immunotherapy Upon Recognition of Deficient Mismatch Repair Signature on Liquid Biopsy: A Case Report." *Cureus.* 14.7 (2022).
16. Komura, A., et al. "A case of refractory pelvic squamous cell carcinoma of unknown primary that responded to nivolumab." *J Obstet Gynaecol Res.* 49.4 (2023): 1300-1304.
17. Laprovitera, N., et al. "Genetic characterization of cancer of unknown primary using liquid biopsy approaches." *Front Cell Dev Biol.* 9 (2021): 666156.
18. Kolbinger, F.R., et al. "Significance of distinct liquid biopsy compartments in evaluating somatic mutations for targeted therapy selection in cancer of unknown primary." *J Gastrointest Cancer.* 54.4 (2023): 1276-1285.
19. Conway, A.M., et al. "A cfDNA methylation-based tissue-of-origin classifier for cancers of unknown primary." *Nat Commun.* 15.1 (2024): 3292.
20. De W.J., et al. "A fast, affordable and minimally-invasive diagnostic test for Cancer of Unknown Primary (CUP) using DNA methylation profiling." *Lab Investig.* (2024): 102091.
21. Krämer, A., et al. "Comprehensive profiling and molecularly guided therapy (MGT) for carcinomas of unknown primary (CUP): CUPISCO: A phase II, randomised, multicentre study comparing targeted therapy or immunotherapy with standard platinum-based chemotherapy." *Ann Oncol.* 29 (2018): 146.
22. Sultova, E. *Implementation of precision medicine for patients with metastatic breast cancer in an interdisciplinary MTB setting. Diss. Lmu.* 2024.
23. Laprovitera, N., et al. "Cancer of unknown primary: challenges and progress in clinical management." *Cancers.* 13.3 (2021): 451.
24. Conway, A.M., et al. "Molecular characterisation and liquid biomarkers in Carcinoma of Unknown Primary (CUP): taking the 'U'out of 'CUP'." *Br J Cancer.* 120.2 (2019): 141-153.
25. Bardelli, A., & Klaus P. "Liquid biopsies, what we do not know (yet)." *Cancer Cell.* 31.2 (2017): 172-179.
26. Kagawa, Y., et al. "Combined analysis of concordance between liquid and tumor tissue biopsies for RAS mutations in

colorectal cancer with a single metastasis site: the METABEAM study." *Clin Cancer Res.* 27.9 (2021): 2515-2522.

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