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ORIGINAL ARTICLE

Clinical utility of comprehensive liquid molecular profiling in patients with advanced endometrial cancer

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Abstract

Background: Molecular characterization has significantly improved the management of advanced endometrial cancer (EC). It distinguishes four molecular subclasses associated with prognosis and personalized therapeutic strategies. This study assesses the clinical utility of cell-free DNA (cfDNA) profiling in EC to identify targetable alterations.

Methods: Women with metastatic or recurrent EC were prospectively recruited within the framework of the STING trial (NCT04932525), during which cfDNA was analyzed. Genomic alterations were identified with the FoundationOne CDx assay. Each molecular report underwent review by a molecular tumor board. Alterations were categorized via the European Society of Medical Oncology Scale for Clinical Actionability of Molecular Targets (ESCAT).

Results: A total of 61 patients were enrolled. The median age was 66.9 years, with 43% presenting frontline metastatic disease. All histologic subgroups were represented. Notably, 89% of patients yielded informative cfDNA analysis. Six tumors were classified with deficient mismatch repair/microsatellite instability (11%) and 37 as *TP53* gene mutant (67%), and 12 had nonspecific molecular profiles (22%). Molecular classification based on liquid biopsy showed 87.5% accuracy in correlating with tissue results. Moreover, 65% of cases exhibited ≥ 1 actionable alteration, including 25% ESCAT I alterations and 13% ESCAT II alterations. Consequently, 16% of patients received a molecularly matched therapy, and presented with a 56% response rate and median progression-free survival of 7.7 months.

Conclusions: cfDNA sequencing in EC is a feasible approach that produces informative results in 89% of cases and accurately categorizes patients into the main

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molecular subclasses. It also reveals multiple actionable alterations, which offers the potential for personalized therapeutic strategies.

KEYWORDS

cell-free DNA (cfDNA), clonal hematopoiesis, endometrial cancer, molecular profile, personalized treatment

INTRODUCTION

In recent decades, the incidence and mortality of endometrial cancer (EC) have been on the rise, primarily as a result of a concerning increase in risk factors such as obesity, metabolic syndrome, and diabetes.¹ Although the majority of EC cases are diagnosed at a localized stage, often curable with a combination of surgery, radiation therapy, and cytotoxic platinum-based chemotherapy, approximately 20% of newly diagnosed cases present as advanced/metastatic disease carrying a grim prognosis.^{2,3}

The molecular classification of EC by The Cancer Genome Atlas program revealed four distinct molecular subgroups of EC based on copy-number alterations (CNAs) and tumor mutational burden (TMB).⁴ These subgroups are as follows: (1) *POLE*-mutated (*POLEmut*) EC, (2) EC with microsatellite instability and a deficient mismatch repair pathway (dMMR/MSI), (3) a subgroup of EC with low CNAs and TMB and a stable microsatellite status (CN-low), and (4) a CN-high subgroup with a high number of CNAs and a low mutational rate. It is strongly linked to nonendometrioid histology, particularly serous, and *TP53* mutations. Importantly, this classification holds significant prognostic relevance, with *POLEmut* EC demonstrating excellent outcomes, dMMR/MSI and CN-low EC displaying stage-dependent intermediate outcomes, and CN-high EC having the poorest clinical outcomes. Consequently, molecular classification has been incorporated into the diagnostic algorithm for guiding decisions regarding adjuvant chemoradiation, and several active clinical trials are now offering personalized strategies based on these molecular subgroups to improve cure rates and reduce toxicity.⁶⁻⁸

Recently, the management of advanced/metastatic EC has been greatly improved because of this molecular characterization. In cases of dMMR/MSI advanced EC, which accounts for approximately 30% of cases, immune-checkpoint inhibitors (programmed death 1 [PD-1]/programmed death ligand 1 [PD-L1] inhibitors) have shown remarkable response rates and significantly prolonged survival outcomes.^{9,10} As a result, PD-1/PD-L1 inhibitors have become part of international recommendations for the treatment of advanced/metastatic dMMR/MSI EC.^{11,12} Similarly, *POLEmut* EC is suspected to be highly responsive to immunotherapy, although there is a lack of prospective data because of its rarity in the metastatic setting.¹³ Finally, there are several other promising targets for personalized therapeutic strategy in advanced EC, including *ERBB2* amplifications, frequently detected in *TP53* mutant (*TP53mut*) EC,¹⁴ *FGFR2* mutations,¹⁵ *CCNE1* amplifications,¹⁶ and homologous recombination (HR) repair alterations.¹⁷

In the context of metastatic solid tumors, circulating cell-free DNA (cfDNA) profiling has proven to be acceptable and clinically valuable for detecting molecular alterations and guiding treatment

decisions.^{18,19} However, despite the increasing importance of EC molecular profiling, clinicians often face challenges in obtaining sufficient tissue for large panel sequencing. Remarkably, there are only very limited data regarding the clinical utility of comprehensive liquid molecular profiling in advanced EC.^{20,21} We aim to report, for the first time, the clinical value of prospective analysis of cfDNA in a cohort of patients with advanced EC. In particular, our objectives were to describe the feasibility of cfDNA sequencing analysis performed in patients with advanced EC, and explore its clinical utility in terms of molecular classification, detection of actionable alterations, and benefit of matched therapies.

MATERIALS AND METHODS

Patients

Patients were prospectively enrolled in the STING trial (NCT04932525) at Institut Gustave Roussy (IGR) in France, which allowed the collection of blood samples from patients with advanced solid tumors for cfDNA profiling.

Here, we focus on a cohort of women with recurrent, advanced, or metastatic EC. Patients were eligible for inclusion in the study at any point of their disease history, whether it was before, during, or after systemic treatment, regardless of the prior number of lines. Blood samples for cfDNA analysis were collected on the day of enrollment.

This study was approved by the Ethical Review Board of IGR and conducted in accordance with the Declaration of Helsinki. Written informed consent for translational research substudies was obtained from all participants before enrollment.

Molecular profiling

cfDNA was isolated from plasma derived from anticoagulated peripheral whole blood. Extracted cfDNA underwent hybrid capture-based targeted DNA sequencing with the FoundationOne CDx assay, which covers 324 genes with high-uniform depth (targeting >500× median coverage with >99% of exons at a coverage of >100×) and provides TMB and MSI status. TMB was calculated by counting somatic variants (single-nucleotide and indels, including synonymous variants, excluding germline and driver mutations) with a variant allele frequency (VAF) of $\geq 0.5\%$. MSI score was assessed with >1800 repetitive loci.

Only alterations classified as pathogenic or likely pathogenic were considered in the analysis. Each individual molecular report

underwent comprehensive review and discussion by a molecular tumor board (MTB) consisting of organ specialists, phase 1 specialists, biologists, and geneticists. Pathogenic or likely pathogenic alterations of clonal hematopoiesis (CH) genes evaluated by the panel, including *DNMT3A*, *TET2*, *ASXL1*, *SF3B1*, *JAK2*, *MPL*, *IDH1*, *IDH2*, *U2AF1*, and *MYD88*, were also registered. High-risk CH was defined as mutations in *JAK2*, *MPL*, or *MYD88* regardless of their variant allele fraction, or as mutations in *DNMT3A* or *TET2* or *ASXL1* or *IDH1* or *IDH2* or *SF3B1* or *U2AF1* genes with a VAF of $\geq 10\%$.^{22–24} *TP53* and *ATM* mutations, which frequently occur as EC-related mutations, were not considered related to CH in this analysis.

cfDNA analysis was considered informative if the panel detected at least one alteration (single-nucleotide variant or CNA) not associated with CH. Alterations that were potentially actionable were categorized via the European Society of Medical Oncology Scale for Clinical Actionability of Molecular Targets (ESCAT) tier.²⁵ Patients for whom a targetable alteration was detected had the option to participate in clinical trials running at IGR.¹⁹

Patients could benefit from tumor molecular classification based on tissue analysis as part of their disease management. Molecular classification by tissue was performed with MMR immunohistochemistry (IHC) and p53 IHC or *TP53* sequencing, following international recommendations.⁶ For molecular class assignment the diagnostic Vermij algorithm was used.²⁶ This algorithm also provided support for cases that had more than one classifying feature, sometimes referred to as “double classifiers.” With cfDNA, molecular classification was determined by MSI testing, and *TP53* status was determined by targeted sequencing. Similarly, class assignment was made via the Vermij algorithm. No matched normal DNA was analyzed.

Statistical considerations

Categorical variables are reported as frequencies (percentages) and continuous variables as medians (interquartile ranges). The objective

response rate (ORR) was determined as the percentage of patients achieving either a partial or complete response according to Response Evaluation Criteria in Solid Tumors, version 1.1. Overall survival (OS) was calculated from the date of inclusion to the date of last follow-up or death. Progression-free survival (PFS) was calculated from the date of treatment start to the date of progression or last follow-up if treatment was ongoing. Survival outcomes were generated via the Kaplan–Meier method. The threshold for statistical significance was set at $p < .05$. All statistical analyses were performed with R software, version 4.1.3, and the BlueSky R package, version 10.2.1.

RESULTS

Patients

Between January 2021 and September 2023, 61 patients with advanced EC were prospectively recruited into the STING trial (Figure 1). Among the overall cohort, 26 patients (43%) had frontline metastatic disease, whereas 35 patients (57%) had recurrent disease. The median age was 66.9 years, the distribution of histologic types included 33% serous EC (20 of 61), 29% low-grade and 12% high-grade endometrioid EC, and 18% carcinosarcomas. Forty-six percent of the patients were aged older than 70 years at the time of inclusion, including 12% who were aged older than 80 years. Patient characteristics are summarized in Table 1. The median time between the diagnosis of advanced disease and trial inclusion was 7.7 months, with a range of 2.8–19.7 months (quarters 1–3). With a median follow-up of 11.7 months, OS for the entire cohort was 14.0 months (95% CI, 10.5 months to not achieved [NA]). The 1- and 2-year OS rates were 56.2% (95% CI, 43.1%–73.4%) and 30.3% (95% CI, 16.5%–55.5%).

Patients were sampled before initiation of any systemic treatment in 34% of cases, during an effective systemic therapy in 20% of cases, and at disease progression in 46% of cases (Table 1). A total of 326 alterations were detected, including 47 amplifications, four deletions,

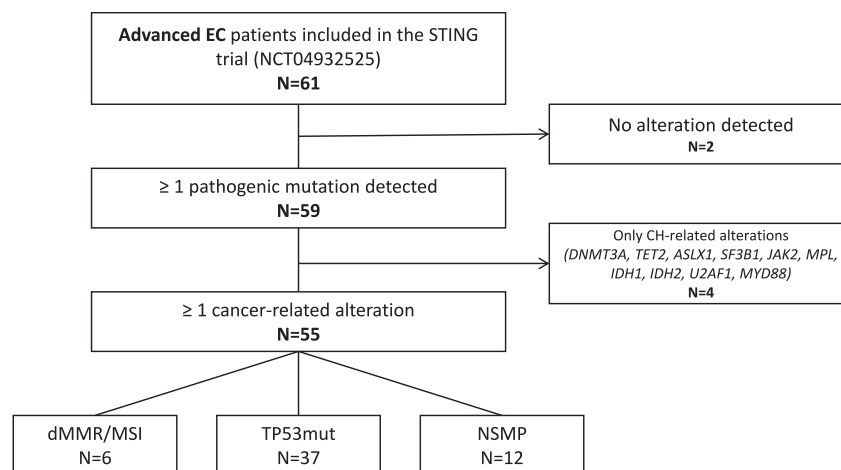


FIGURE 1 Consolidated Standards of Reporting Trials diagram of patients with advanced EC included in the study. CH indicates clonal hematopoiesis; dMMR/MSI, deficient mismatch repair/microsatellite instability; EC, endometrial cancer; NSMP, nonspecific molecular profile; *TP53mut*, *TP53* gene mutation.

TABLE 1 Patient characteristics included in the study.

	Overall (N = 61)
Age at diagnosis, years	
Median (Q1–Q3)	66.9 (60.6–73.3)
FIGO stage (2023) at diagnosis, No. (%)	
I	20 (33.3)
II	3 (5.0)
III	14 (23.3)
IV	23 (38.3)
Frontline advanced versus recurrent disease, No. (%)	
Frontline Metastatic/Advanced	26 (42.6)
Recurrent	35 (57.4)
Histology, No. (%)	
Serous	20 (32.8)
Low-grade endometrioid	18 (29.5)
Carcinosarcoma	11 (18.0)
High-grade endometrioid	7 (11.5)
Undifferentiated	2 (3.3)
Mixed	2 (3.3)
Clear cell	1 (1.6)
Histopathologic grade, No. (%)	
High	43 (70.5)
Low	18 (29.5)
Time point of liquid biopsy, No. (%)	
Before systemic treatment initiation	21 (34.4)
During systemic therapy (PR/SD)	12 (19.7)
Progressive disease	28 (45.9)

Abbreviations: FIGO, International Federation of Gynecology and Obstetrics; PR, partial response; Q, quarter; SD, stable disease.

three fusions, and 272 mutations. The most frequently altered genes were *TP53* (N = 42; 68.9%), *DNMT3A* (N = 23; 37.7%), *PIK3CA* (N = 20; 32.8%), *PTEN* (N = 15; 24.6%), *ERBB2* (N = 9; 14.8%), *CTNNB1* (N = 9; 14.8%), and *PPP2R1A* (N = 8; 13.1%). The main molecular alterations encountered in this cohort are summarized in Figure 2.

Of the 61 patients, 55 (90.2%) achieved an informative cfDNA analysis. Among the six patients (9.8%) with noninformative cfDNA analysis, with no tumor-related driver detectable in their liquid biopsy, four patients were sampled during an effective systemic therapy and one patient had locoregional recurrence without any evidence of distant metastasis.

Molecular classification

According to cfDNA results, 11% of patients (6 of 55) were classified as dMMR/MSI, 67% of patients (37 of 55) were classified as *TP53*mut, and

22% of patients (12 of 55) were classified as nonspecific molecular profile (NSMP). No *POLE* mutations were detected. *TP53*mut EC was the predominant classification among serous tumors, which accounted for 19 of the 20 serous tumors (94%) and the majority of high-grade tumors (81%). In contrast, the dMMR/MSI and NSMP subgroups included various histologic subtypes and grades.

We then sought to compare the molecular classifications based on liquid biopsy and tissue analysis according to Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE) classification.²⁷ Tissue-based molecular classification was available for 54 patients and showed strong concordance with the liquid biopsy results. There were only minor discrepancies: tumors for four patients with an NSMP based on tissue sequencing were classified as *TP53*mut on liquid biopsy and vice versa, two patients with *TP53*mut tumors based on tissue sequencing were classified as NSMP by liquid biopsy (ID 680 and 1557). Details outlining these discrepant cases are provided in Table S1.

Notably, seven patients for whom tissue analysis was not available because of a small biopsy or poor DNA quality had contributive cfDNA sequencing, which allowed molecular classification. Overall, molecular classification based on liquid biopsy had an accuracy rate of 87.5% (42 of 48) for predicting tissue-based results. Molecular classification based on cfDNA and tissue analysis is summarized in Figure 2.

Actionable targets

Of the 55 patients with contributive cfDNA analysis, 35 patients (63.6%) exhibited at least one actionable alteration. We detected 25% of patients (14 of 55) with an ESCAT I alteration, 13% of patients (7 of 55) with an ESCAT II alteration, and 25% of patients (14 of 55) with an ESCAT IIIA alteration. The list of targetable alterations classified according to the ESCAT system is summarized in Table 2.

ESCAT I alterations

dMMR/MSI was detected in cfDNA from six patients (11%), and was always associated with high TMB as defined by >15 mutations per megabase. The dMMR phenotype was associated with multiple pathogenic mutations in *TP53*, *PTEN*, and *PIK3CA* genes, among others.

ERBB2 amplification was found in the liquid biopsy of seven patients (13%), all with *TP53*mut EC. Half of the *ERBB2*-amplified ECs were serous (four of seven; 57.1%), two patients had carcinosarcomas, and one patient had a low-grade endometrioid EC. Last, two patients had *FGFR2* mutations, one of which was associated with dMMR/MSI status.

ESCAT II alterations

ERBB2 mutations were detected in the cfDNA of two patients (3.6%), both of whom had *TP53*mut tumors. Additionally, four patients

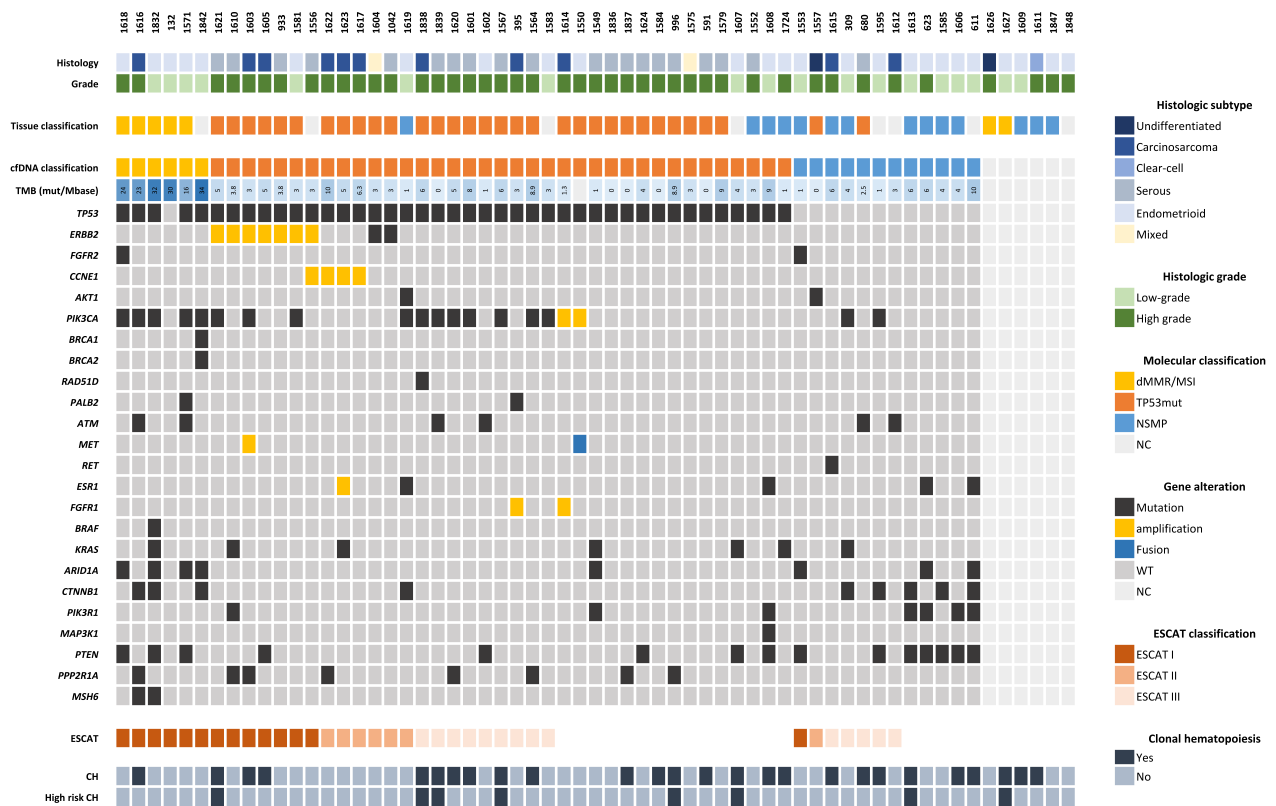


FIGURE 2 Molecular alterations detected on cfDNA analysis and correlation with clinical characteristics and molecular classification based on tissue analysis. cfDNA indicates cell-free DNA; CH, clonal hematopoiesis; dMMR/MSI, deficient mismatch repair/microsatellite instability; ESCAT, ESMO Scale of Clinical Actionability for Molecular Targets; NC, noncontributive; NSMP, nonspecific molecular profile; TMB, tumor mutational burden; *TP53mut*, *TP53* gene mutation; WT, wild type.

presented with *CCNE1* amplification on the basis of cfDNA analysis (7.3%). These amplifications were exclusively observed in *TP53mut* EC, including three carcinosarcomas and one serous tumor. Finally, two patients presented with a tumor with an *AKT1*^{E17K} mutation, one patient with a low-grade endometrioid NSMP tumor and the other with an undifferentiated NSMP carcinoma.

ESCAT III alterations

Twenty patients presented with *PIK3CA* alterations, including two amplifications and 18 mutations. Recurrent pathogenic variants were detected, including *PIK3CA*^{H1047R} in four cases and *PIK3CA*^{E454K}, *PIK3CA*^{E81K}, and *PIK3CA*^{G118D} in two cases each. The HR repair pathway was frequently mutated in the cohort, with 11 mutations detected across nine different patients (nine of 54; 16%). These included one *RAD51D*, two *PALB2*, and six *ATM* mutations. The low allelic frequency observed in HR gene mutations excluded a germline origin for all these patients. One patient with a dMMR/MSI tumor had concurrent *BRCA1* and *BRCA2* mutations.

Interestingly, four patients in our cohort showed *ESR1* mutations, including two *ESR1*^{Y537S} mutations. All of these patients had received and progressed under endocrine therapy before cfDNA analysis. Three received an aromatase inhibitor and one received progestogen therapy and remained under treatment for 9, 11, 12, and 15 months, respectively.

Among the other ESCAT III alterations, various others were identified, including one *ST7-MET* fusion, one *MET* amplification, one *FGFR1* amplification, and one *RET* mutation (*RET*^{E511K}).

Of the patients with contributive results, nine patients (16.4%) received genomically matched therapy on the basis of an MTB recommendation. Four patients received a PD-1/PD-L1 inhibitor, either as monotherapy or in combination with lenvatinib, another checkpoint inhibitor, or a PARP inhibitor. Two patients received anti-*ERBB2* therapy, and three received a targeted therapy including *FGFR*, *MET*, and *PARP* inhibitors. These targeted therapies were chosen on the basis of specific alterations identified in the cfDNA analysis. Six patients received the therapy as part of a clinical trial, whereas two received it as standard therapy and one received the matched-treatment off-label. In our study, the ORR with matched

TABLE 2 List of genomic alterations potentially actionable on the basis of their level of evidence detected by cfDNA next-generation sequencing.

ESCAT alteration	Molecular alteration on cfDNA	ESCAT tier	Incidence, No. (%)	Reference
I	dMMR/MSI	IA	6 (11.3)	9,10,28,29
	<i>ERBB2</i> amplification	IA	7 (13.2)	30,31
	<i>FGFR2</i> mutation	IC	2 (3.8)	32
II	<i>ERBB2</i> mutation	IIB	2 (3.8)	33
	<i>AKT1</i> mutation	IIB	2 (3.8)	34
	<i>CCNE1</i> amplification	IIB	4 (7.5)	35
III	<i>PIK3CA</i> mutation	IIIA	18 (34.0)	36
	<i>BRCA1</i> mutation	IIIA	1 (1.9)	37
	<i>BRCA2</i> mutation	IIIA	1 (1.9)	37
	<i>RAD51D</i> mutation	IIIA	1 (1.9)	38
	<i>PALB2</i> mutation	IIIA	2 (3.8)	39
	<i>ATM</i> mutation	IIIA	6 (11.3)	40
	<i>RET</i> mutation	IIIA	1 (1.9)	41
	<i>ESR1</i> mutation	IIIA	4 (7.5)	42
	<i>MET</i> amplification	IIIA	1 (1.9)	43
IV	<i>KRAS</i> G12A, G12D, G12R, and G12V	IV	5 (9.4)	44,45
	<i>BRAF</i> non-V600 mutation	IV	1 (1.9)	46
	<i>ARID1A</i> mutation	IV	8 (15.1)	47
	<i>SMARCA4</i> mutation	IV	2 (3.8)	48
	<i>CDH1</i> mutation	IV	2 (3.8)	49
	<i>PIK3R1</i> mutation	IV	7 (13.2)	50
	<i>MAP3K1</i> mutation	IV	1 (1.9)	51
	<i>ST7-MET</i> fusion	IV	1 (1.9)	52
<i>FGFR1</i> amplification	IV	2 (3.8)	53	

Note: Alterations were categorized via ESCAT tier.²⁵

Abbreviations: cfDNA, cell-free DNA; dMMR/MSI, deficient mismatch repair/microsatellite instability; ESCAT, ESMO Scale for Clinical Actionability of Molecular Targets.

therapy was 55.6% and median PFS was 7.7 months (95% CI, 3.7 months to NA). Details are provided in Table S2.

CH

Of the overall cohort, 26 patients (42.6%) had at least one alteration in a CH gene identified by cfDNA sequencing. These CH gene mutations had a median VAF of 1.1% with an interquartile range of 0.47%–3.92%, and 11 patients (18.0%) had a CH mutation with a VAF greater than or equal to 2%. In total, eight patients carried at least one high-risk CH mutation (13.1%). The median OS for patients with high-risk CH was 7.8 months (95% CI, 7.8 months to NA). In contrast, patients without high-risk CH had a median OS of 14.4 months (95% CI, 10.5 months to NA). However, the difference was not statistically significant, with a *p* value of .284.

DISCUSSION

This study presents a large cohort of patients with advanced EC who underwent prospective cfDNA sequencing analysis. As with other tumor types, we have demonstrated the feasibility of liquid biopsy in advanced EC, with contributive results obtained in more than 88% of cases.¹⁹ In this study, cfDNA results from 61 patients accurately categorized patients into the main EC molecular subgroups, including the dMMR/MSI subgroup. Importantly, they provided molecular classification for patients with noncontributive tissue analysis. Specifically, liquid biopsy showed high accuracy compared to tissue classification based on IHC or sequencing assays.⁵⁴ Moreover, cfDNA provides a tool to characterize metastatic disease without the need for a new tumor biopsy.

Our findings align with the known molecular characteristics of EC published in the literature. First, *TP53* mutations are frequent in

metastatic/relapsed EC, being the most common alteration in our cohort, detected in almost 70% of the patients. The *TP53*mut subgroup comprises most nonendometrioid subtypes (type 2 tumors) and is associated with a poorer prognosis.^{4,5,55,56} We further confirmed that *TP53*mut EC displays specific clinically valuable molecular alterations, such as *ERBB2* alterations, *CCNE1* amplifications, and *RAD51D* and *PALB2* mutations.^{16,17,26} Second, the PI3K/AKT/mTOR pathway is frequently altered in our cohort, primarily because of *PIK3CA* or *PTEN* mutations, detected in 33% and 25% of cases, respectively. Notably, these alterations cut across all histologies and molecular subgroups. This high incidence highlights the potential of novel agents targeting this pathway, such as AKT inhibitors, which are currently under investigation.⁵⁷ Last, dMMR/MSI EC represents a minority of advanced EC cases, accounting for 15%–30%.^{4,58} Here, we confirm that cfDNA is a valuable tool for detecting MSI, even when tissue analysis yields noncontributive results.

The extensive molecular profiling in our cohort revealed numerous potentially targetable alterations. Remarkably, 64% of patients with EC exhibited at least one actionable alteration, which emphasizes the importance of systematic large-panel sequencing, whether it is conducted on tumor tissue or cfDNA.⁵⁸ Twenty-five percent of metastatic EC tumors harbored an ESCAT I alteration, and 13% harbored an ESCAT II alteration. In this context, appropriately targeted therapies have the potential to provide clinical benefits and should be considered as a promising therapeutic option.²⁵ Furthermore, approximately 25% of the cohort displayed ESCAT III alterations. Discussions regarding participation in clinical trials should be considered for these patients. Interestingly, 16% of patients had HR gene mutations in cfDNA, which mainly involved *ATM*. One patient with known dMMR harbored concomitant *BRCA1* and *BRCA2* mutations; however, because these are simply a consequence of hypermutability, it is unclear whether they would induce HR deficiency or PARP inhibitor sensitivity. In our study, treatment with a matched therapy resulted in an impressive 56% ORR and 7.7-month PFS. Unfortunately, only nine patients received matched therapies. One explanation may be that patients with EC are often relatively fragile. Therefore, subsequent lines of treatment, especially inclusion in clinical trials, can be challenging. These observations underscore the importance of early molecular profiling when patients are still relatively fit, and advocate for dedicated trials tailored to the unique context of patients with EC. Finally, hormonal therapies remain a cornerstone of management for advanced EC, and the detection of *ESR1* mutations in four patients previously treated with hormonal agents suggests that these mutations could account for resistance. This could provide crucial information to guide subsequent therapy with estrogen receptor degraders, for example.

Interestingly, cfDNA not only provides insights into the tumor's molecular background but also detects high-risk CH.²³ In our cohort, 13% of patients presented with at least one high-risk alteration, likely due to factors such as aging or exposure to external mutagens. This

suggests a 5% estimated risk of developing a hematologic malignancy.^{59,60} Furthermore, these patients tend to exhibit lower OS compared to those without high-risk CH. In such cases, the MTB recommends seeking advice from hematologists. The benefits of early intervention in these cases remain an area of investigation. Approximately 43% of the cohort manifested at least one CH-associated alteration. This prevalence aligns with expectations for an elderly population pretreated with systemic chemotherapy.⁵⁴

Our study carries several limitations. First, tissue-based sequencing was not performed in all patients, which makes it challenging to perform a direct comparison of liquid and tissue molecular profiles. Among those with paired tissue and liquid analysis, six patients exhibited discrepancies. One explanation is that cfDNA reflects the current molecular state of the cancer, particularly the subpopulation of circulating tumor cells, which may exhibit a different molecular profile compared to the historical tumor. Conversely, these discrepant results could indicate that cfDNA primarily reflects CH with no traces of tumor cells (ID 1619, 1724, and 680; Table S1). Specifically, *TP53*, *ATM*, and *CHEK2* mutations, which frequently occur either as CH or cancer-related mutations, may have misled molecular classification and MTB interpretation.²³ cfDNA is considered a surrogate biomarker for tumor burden, and is closely associated with outcomes.^{61,62} Thus, noncontributive results may also reflect the paucity of cfDNA shedding, particularly in cases of local relapses or concomitant effective systemic treatment. Indeed, among the 12 patients sampled during an effective therapy, four had noncontributive cfDNA sequencing (33%). In this context, although this study was not designed to compare the different time points of sampling, cfDNA sequencing should probably be considered at disease recurrence/progression.

In conclusion, cfDNA sequencing in advanced EC is feasible, and provides contributive results in 90% of cases and accurate categorization of patients into the main molecular subclasses. In addition, EC may be particularly suited to this approach because potentially actionable alterations were identified in 64% of patients, which suggests a potential benefit from matched therapy. Last, cfDNA unveils high-risk CH, for which dedicated intervention may prove beneficial for the patient. Overall, cfDNA profiling serves as a valuable tool for tailoring the management of patients with metastatic EC.

AUTHOR CONTRIBUTIONS

Félix Blanc-Durand: Conceptualization, investigation, validation, methodology, writing—original draft, writing—review and editing, and formal analysis. **Geraldine M. Camilleri:** Data curation and writing—review and editing. **Arnaud Bayle:** Data curation. **Mihaela Aldea:** Writing—review and editing and supervision. **Damien Vasseur:** Writing—review and editing and supervision. **Kaissa Ouali:** Writing—review and editing and supervision. **Judith Michels:** Supervision. **Patricia Pautier:** Supervision. **Claudio Nicotra:** Project administration. **Maud Ngo-Camus:** Project administration. **Ludovic Lacroix:**

Formal analysis and supervision. **Etienne Rouleau**: Formal analysis and supervision. **Santiago Ponce-Aix**: Supervision. **Antoine Italiano**: Supervision. **Alexandra Leary**: Conceptualization, writing-review and editing, validation, and supervision.

CONFLICT OF INTEREST STATEMENT

Arnaud Bayle has been a consultant for F. Hoffmann-La Roche and Sanofi and received travel support from Pfizer. Judith Michels has received grants from Merck Sharp & Dohme and been a consultant for Regeneron Pharmaceuticals and Brenus Pharma. Etienne Rouleau has been a consultant for Clovis Oncology, Roche Diagnostics, Bristol-Myers Squibb, GlaxoSmithKline, and AstraZeneca. Antoine Italiano has received grants from F. Hoffmann-La Roche, Merck, AstraZeneca, Chugai Pharmaceutical, Bayer, and Bristol-Myers Squibb and been a consultant for Chugai Pharmaceutical. Alexandra Leary has received institutional grant funding from Merck Sharp & Dohme, Zentalis Pharmaceuticals, and GlaxoSmithKline and been a consultant for AstraZeneca. The other authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available because of privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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