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Research paper

Circulating tumor DNA monitoring for early recurrence detection in epithelial ovarian cancer

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HIGHLIGHTS

- Post-surgical ctDNA detection is prognostic of reduced recurrence-free survival.
- Post-surgically, ctDNA detected relapse with 100% sensitivity and specificity.
- ctDNA detection preceded radiological findings by an average lead time of 10 months.
- The presence of ctDNA and not CA-125 was a strong predictor of relapse.

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ABSTRACT

Objective. Epithelial ovarian cancer (EOC) is the most lethal gynecologic malignancy. We examined the utility of circulating tumor DNA (ctDNA) as a prognostic biomarker for EOC by assessing its relationship with patient outcome and CA-125, pre-surgically and during post-treatment surveillance.

Methods. Plasma samples were collected from patients with stage I-IV EOC. Cohort A included patients with pre-surgical samples ($N = 44$, median follow-up: 2.7 years), cohort B and C included: patients with serially collected post-surgically ($N = 12$) and, during surveillance ($N = 13$), respectively (median follow-up: 2 years). Plasma samples were analyzed using a tumor-informed, personalized multiplex-PCR NGS assay; ctDNA status and CA-125 levels were correlated with clinical features and outcomes.

Results. Genomic profiling was performed on the entire cohort and was consistent with that seen in TCGA. In cohort A, ctDNA-positivity was observed in 73% (32/44) of presurgical samples and was higher in high nuclear grade disease. In cohort B and C, ctDNA was only detected in patients who relapsed (100% sensitivity and specificity) and preceded radiological findings by an average of 10 months. The presence of ctDNA at a single timepoint after completion of surgery +/− adjuvant chemotherapy and serially during surveillance was a strong predictor of relapse (HR:17.6, $p = 0.001$ and $p < 0.0001$, respectively), while CA-125 positivity was not ($p = 0.113$ and $p = 0.056$).

Conclusions. The presence of ctDNA post-surgically is highly prognostic of reduced recurrence-free survival. CtDNA outperformed CA-125 in identifying patients at highest risk of recurrence. These results suggest that monitoring ctDNA could be beneficial in clinical decision-making for EOC patients.

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1. Introduction

Epithelial ovarian cancer (EOC) is the most lethal gynecologic malignancy worldwide [1]. In the U.S., it is the fifth leading cancer type in women, accounting for 5% of overall cancer-related deaths, with an estimated 19,880 new cases and 12,810 deaths in 2022 alone [2]. The poor survival is largely driven by the advanced stages (III/IV) at which EOC is diagnosed [3]. The 5-year survival rate decreases markedly with increase in stage (stage I: 90%, stage II: 0–40%, stage III: 15–20%, stage IV: <5%) [4] with the majority of patients recurring, despite achieving complete remission with primary treatment [5].

The current standard of care (SOC) involves a combination of surgical debulking and platinum-based chemotherapy for patients with EOC [4]. Classically, stage, cancer antigen-125 (CA-125) levels, radiologic response, and presence of residual disease following cytoreductive surgery were the main factors used for decision making regarding duration of therapy and use of maintenance therapy. Numerous maintenance strategies have been evaluated, including systemic chemotherapy [5] and vascular endothelial growth factor (VEGF) inhibition [6]. More recently, poly (ADP-ribose) polymerase inhibitors (PARPi) have been introduced into the maintenance and treatment paradigm [7,8]. The magnitude of benefit with these strategies varies based on patient, pathologic, and molecular characteristics.

While the approach to treatment selection has evolved, tools for prognosis and prediction of treatment benefit have not. CA-125 levels, physical exam, and imaging remain the standard for monitoring response to treatment and detecting relapse [9]. Approximately 80% of EOC will have elevated levels of CA-125 at diagnosis. While CA-125 specificity for detection of recurrence is high (91–100%) with a lead time between 2 and 5 months, sensitivity varies widely (56–94%) [10–14]. Similar wide ranges have been seen with computed tomography (CT), with sensitivity ranging from 47 to 75% and specificity ranging from 78 to 87% [13,15]. Furthermore, neither imaging nor CA-125 for early detection of recurrence has been found to improve clinical outcomes.

Circulating tumor DNA (ctDNA) has emerged as a non-invasive biomarker that can detect residual disease at a molecular level months ahead of radiological findings. Here, we investigated the clinical validity of ctDNA for molecular residual disease (MRD) detection and to predict recurrence during surveillance. We evaluated the association of ctDNA-based MRD status with recurrence-free survival (RFS) and overall survival (OS, subset of Cohort A) in this study We also provide evidence that detection of ctDNA demonstrates higher sensitivity and specificity for recurrence than monitoring CA-125 levels and precedes radiological findings.

2. Methods

2.1. Patients

This study included patients diagnosed with stage I-IV EOC from University of California San Francisco (Cohort A), Columbia University Irving Medical Center, University of Pittsburgh, and Stanford University (Cohorts B and C). Archival plasma samples from pre-surgery were available for Cohort A. Samples for cohorts B and C were serially collected after surgery and during surveillance, respectively. This study was conducted in accordance with the principles of the Declaration of Helsinki and ICH guidelines for Good Clinical Practices. Written informed consent was obtained from all participants included in the study, prior to their enrollment. The study was approved by the corresponding Internal Institutional Review Boards/Ethical Committees (protocol # AAAQ9869, 2016-437A and 33,540).

Cohort A included patients with either known ovarian, fallopian tube, or peritoneal cancer or had an adnexal mass suspicious for malignancy on imaging and ultimately found to be malignant. Cohorts B and C consisted of patients with ovarian, fallopian tube, or peritoneal cancers enrolled following cytoreductive surgery (Cohort B; adjuvant setting) or after the completion of adjuvant therapy (Cohort C; surveillance setting). Patients in cohorts B and C were followed up every 3 months for up to 40 months after the end of definitive therapy. In cohort B, the first blood draw was collected 30 days after surgery. Blood samples were collected at routine follow-up clinical visits.

All patients received treatment and follow-up in compliance with the standard clinical practice, according to the primary oncologist's choice and in the best interest of the patient. Collected samples were analyzed retrospectively. Natera performed ctDNA analysis and remained blinded to the clinical outcomes until reporting results to investigators.

2.2. Biospecimen collection and processing

Pre-surgical blood samples were collected in K2-EDTA 10 ml tubes (Becton Dickinson) at UCSF. All blood samples were processed by double centrifugation at room temperature, first for 10 min at 3000*g*, followed by centrifugation of plasma for 10 min at 3000*g*. Plasma was aliquoted into 5 ml cryotubes and stored at −80 °C. Up to 10 ml of plasma per case was used for this study (range, 0.8–10 ml; median 4.3 ml) and cfDNA was extracted using the QIAamp Circulating Nucleic Acid kit (Qiagen) and eluted into 50 μl DNA Suspension Buffer (Sigma). Each cfDNA sample was quantified by Quant-iT High Sensitivity dsDNA Assay Kit (Invitrogen).

For patients with longitudinal blood samples a CLIA-validated Standard Operating Procedure was used. Specifically, tumor DNA was extracted from formalin-fixed and paraffin-embedded (FFPE) tissue from the resected primary tumor for all patients. Blood samples were collected once in a single 6 ml EDTA test tube for germline DNA analysis and blood samples for cfDNA analysis were collected in two 10 ml Streck tubes every 4 weeks, or at the time of imaging for patients off treatment.

2.3. Development of tumor informed, personalized ctDNA assays

A personalized, tumor-informed, multiplex (m) PCR next generation sequencing (NGS) assay (Signatera™) was used for ctDNA detection and quantification, as previously described.[26] Briefly, whole exome sequencing (WES) was performed on formalin fixed paraffin embedded (FFPE) tumor blocks and matched normal DNA blood samples. Libraries were prepared using a median of 500 ng DNA and captured using targeted exome capture with a custom capture probe set targeting ~20,000 genes. Libraries were then sequenced to achieve the deduplicated on-target average coverage of >180× for tumor tissue and 50× for the associated matched normal sample. FastQ files were prepared using bcl2fastq2 and quality-checked using FastQC. Reads were mapped to the human reference genome hg19 using the Burrows–Wheeler Alignment tool (v.0.7.12) and quality-checked using Picard and MultiQC.

Based on the tissue sequencing results, 2–16 patient-specific somatic clonal variants were selected for mPCR plasma testing. Subsequently, cell-free DNA (cfDNA) was extracted from a median of 10 ml (range: 0.8–10 ml) of plasma using the QIAamp Circulating Nucleic Acid Kit (QIAGEN). mPCR primers targeting the personalized SNV's were designed, synthesized, and tested on an aliquot of cfDNA library, followed by amplicon-based sequencing and an average NGS depth per amplicon of >100,000× on an Illumina platform as previously described.[26] Plasma samples with at least two variants detected were defined as ctDNA-positive. ctDNA concentration was reported in mean tumor molecules (MTM) per ml of plasma.

2.4. Statistical analyses

Wilcoxon rank sum test was used for continuous variables (e.g., ctDNA levels) and Fisher's exact test for categorical variables (e.g., grade and histology). Survival analyses were performed using

Cox regression and were carried out in STATA 16.1 software (StataCorp). All *p*-values were based on 2-sided testing, and differences were considered significant at $p \leq 0.05$.

3. Results

3.1. Patient cohort

Plasma samples were collected from a total of 69 patients. Histologic subtypes included serous ($N = 37$), clear cell ($N = 9$), endometrioid ($N = 9$), and other ($N = 14$). Median age of the entire cohort was 55.5 years (range 29–82 years). The study included three sub-cohorts (A, B, and C) of patients. Cohort A consisted of 44 patients with only pre-surgical plasma samples with a median follow-up of 2.7 years (range: 0.08–16.7). Cohort B consisted of 12 patients with plasma samples collected post-surgically and serially (every 3 months) during and after adjuvant therapy Cohort C consisted of 13 patients with plasma samples collected serially (every 3 months) following completion of definitive treatment (surgery with/without ACT) irrespective of maintenance therapy. The median follow-up for cohort B and C was 2 yrs. (range: 0.3–4.6 yrs). Additional data regarding clinicopathological features and post-surgical interventions are available in Table 1.

3.2. Genomic profiles of EOC patients

Analysis of tissue whole exome sequencing (WES) data was performed to reveal genomic profile characteristics. The most frequent mutations identified were in the following genes: *TP53, ARID1A, KRAS, PIK3CA, EPPK1, BRCA2, BRAF, ATM, BRCA1, PTEN*, and others associated with cancer pathogenesis (Fig. 1). This profile was consistent with those reported by The Cancer Genome Atlas (TCGA) consortium characteristics for EOC patients https://www.genome.gov/Funded-Programs-Projects/Cancer-Genome-Atlas.

3.3. Association of pre-surgical ctDNA detection with clinical features and patient outcomes (Cohort A)

In patients with pre-surgical plasma samples (Cohort A, $n = 44$), high-grade serous ovarian cancer (HGSOC) was the most common histological subtype 66% (29/44), followed by the endometrioid (11%; 5/44), clear cell (7%, 3/44), and other epithelial subtypes (16%; 7/44). ctDNA was detected in 73% (32/44) of pre-surgical samples with detection rates of 69% (20/29) for HGSOC, 80% (4/5) for endometrioid, and 100% (3/3) for clear cell histologies (Fig. 2A). ctDNA prevalence and levels were more elevated in high-grade disease (Fig. 2B). Higher ctDNA-positivity rates were also observed in advanced-stage (Stage III/IV) vs. early-stage (Stage I/II disease (72% vs. 60%, respectively), however, the association was not statistically significant ($p = 0.1$). In addition, pre-surgical ctDNA detection rates and levels (MTM/ml) were elevated in patients who experienced disease progression and died; (ctDNA levels: $p = 0.026$) (Fig. 2C). A total of 41 patients from Cohort A had clinical follow-up available (excluding the two patients that received neoadjuvant therapy). In the ctDNA-positive group ($n = 28$), 43% of patients died of cancer compared to 18% in the ctDNA-negative group ($n = 11$) (Fig. 2D). Median time to death was 2.5 years (range: 0.08–11.5).

Table 1
Patient Demographics and Baseline Characteristics.

Patient characteristics	All patients $n = 69$, (%)
Median Age (at diagnosis)	Median: 55.5 years (range 29–82 years)
Subtype	
Serous	37 (54%)
Clear Cell	9 (13%)
Endometrioid	9 (13%)
Other	14 (20%)
Stage	
I	17 (25%)
II	12 (17%)
III	26 (38%)
IV	3 (4%)
Unstaged	11 (16%)
Origin	
Ovary	64 (93%)
Fallopian Tube	3 (4.3%)
Uterus	2 (3%)
Treatment	
NACT + IDS	2 (3%)
PDS +/-ACT	44 (64%)
Unknown	23 (33%)

Abbreviations: NACT - neoadjuvant therapy, IDS - interval debulking procedure, PDS - primary debulking procedure, ACT - adjuvant chemotherapy.

3.4. ctDNA detection rates after surgical debulking and prior to adjuvant therapy (Cohort B)

In cohort B, post-surgical ctDNA was detectable in 33% (4/12) of patients (Fig. 3A). Of these, 75% (3/4) of patients experienced radiologic recurrence within a median time to recurrence of 19 months (range: 11–37) while only 13% (1/8) MRD-negative patients relapsed. MRD-positive patients had a higher rate of recurrence, although the association did not reach statistical significance (Fig. 3C). Only 3 patients with plasma samples available before and after completion of adjuvant chemotherapy (ACT) were MRD positive. Of these 3 patients, only one cleared ctDNA during ACT with no evidence of disease on imaging, while the other two remained positive and eventually progressed.

3.5. ctDNA detection rates following the completion of adjuvant therapy (Cohorts B and C)

A total of 22 patients from cohorts B and C had ctDNA testing following completion of primary therapy, whether surgery only or ACT (Fig. 3A, B). Of these, 23% (5/22) of patients had detectable ctDNA after completion of definitive therapy, all of whom experienced disease progression (HR: 17.6, 95%CI: 3.2–97.4, $p < 0.001$) (Fig. 3D). Inclusive of additional two patients who became ctDNA-positive during surveillance (32%; 7/22), clinical progression was ultimately confirmed by imaging ($p < 0.0001$) (Fig. 3 E). Longitudinally, ctDNA was detected in patients who experienced relapse with 100% sensitivity (7/7) and 100% specificity (15/15).

Signatera collection was continued on 4 patients with recurrence. Of these 3 had imaging assessment at the time of last follow up. Two of these remained ctDNA-negative after initiation of therapy and it correlated with response by imaging. One patient experienced a transient clearance and ctDNA became detectable 6 months prior to disease progression.

For patients from Cohorts B and C, we conducted a sub analysis of patient-specific variants monitored in plasma to identify driver variants implicated in EOC. The list of identified variants is provided in Supplementary Table 1. Out of 7 relapsed patients, 5 had at least one driver variant included in personalized ctDNA assay. While all the relapsed cases ($n = 5$; 100% sensitivity) were identified with a personalized ctDNA assay longitudinally, only 2 (40%) patients had at least one driver variant detectable in plasma at the time of disease progression (Supplementary Fig. 1).

3.6. ctDNA, CA-125, and routine clinicopathological risk factors to predict risk of recurrence

On comparing CA-125 status with ctDNA to predict risk of recurrence, association of CA-125 levels post-surgery, after ACT, or longitudinally during surveillance were not prognostic in cohort B and C (Fig. 4A). Furthermore, ctDNA was detectable on average 10 months (range: 0–36 months) prior to clinical progression, while CA-125 levels

Fig. 1. Genetic alterations most frequently observed in ovarian cancer patients. Individual cases ($n = 65/69$ patients had variants typically associated with ovarian cancer) grouped by histological subtype are represented on the X axis, mutated genes on the Y-axis. The bar graph above shows the TMB (tumor mutational burden) for each patient, the bar graph on the right represents the mutation frequency of each gene in this cohort. Green represents nonsense mutations, red – in frame indels, yellow – frameshift indels, blue – missense mutations, orange – splice site and purple – silent variants.

had a mean lead time of only ~1 month (range: 0–17) (Wilcoxon signed rank test; $p < 0.05$) (Fig. 4B).

We further assessed association of several clinicopathological risk factors with RFS. After definitive therapy, ctDNA status was compared with CA-125, histology (clear cell vs other), and stage (III, IV vs I, II) using univariate and bivariate Cox regression analyses. Stage and ctDNA status were independently and significantly associated with RFS ($p = 0.021$ and $p = 0.003$) and remained strong predictors in bivariate analysis (adjusted HR: 19, 95%CI: 1.5–236.1, $p = 0.022$, and adjusted HR: 14.5, 95% CI: 1.3–156.2, respectively). We did not observe significant association between CA-125 status or histology with RFS in this dataset (Fig. 4C).

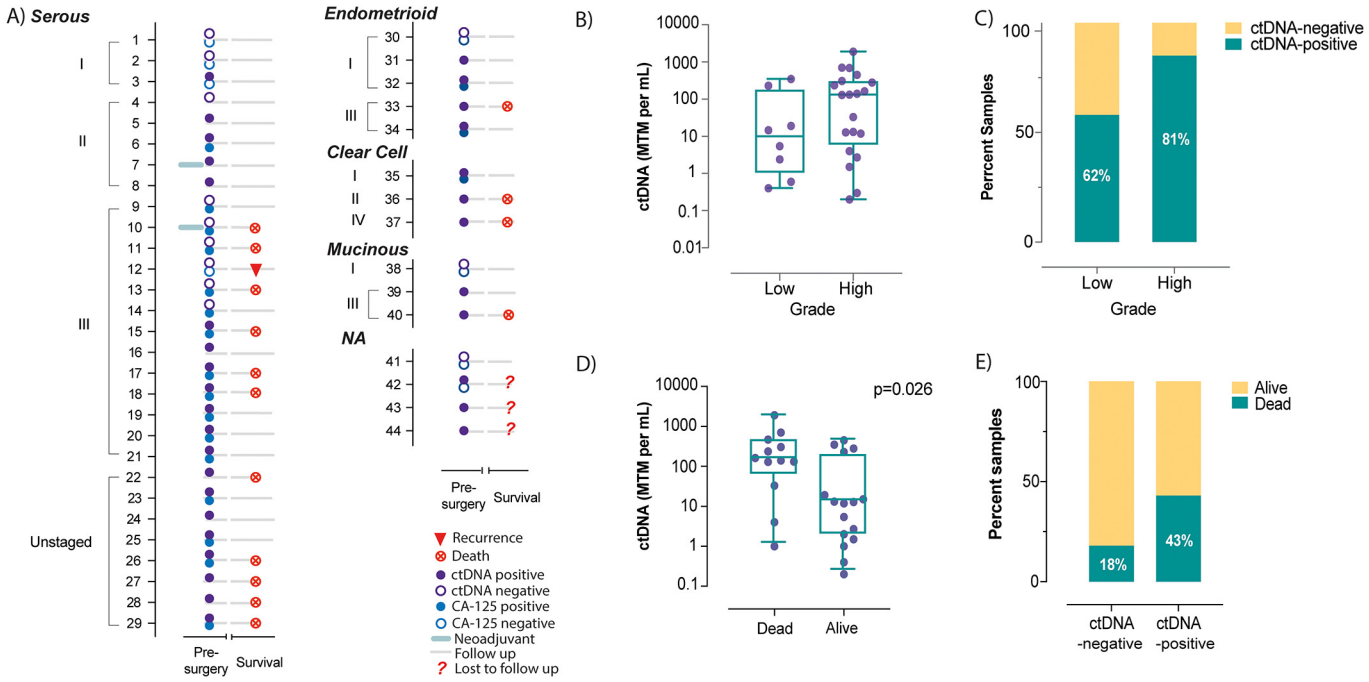


Fig. 2. A) Patient overview plot representing association of pre-surgical ctDNA detection with clinical features and patient outcomes. B-E) Pre-surgical ctDNA detection rates and levels and association with grade and disease progression (Mann Whitney test). For figs. B and C, High represents Grade 3, Low represents Grade 1 and 2. The median follow-up for Fig. 2 (D, E) in the ctDNA-negative group was 2.9 years (range: 0.5–16.7 years), and for the ctDNA-positive group was 2.5 years (range: 1.6–11.5 years). MTM/ml indicates mean tumor molecules per ml of plasma.

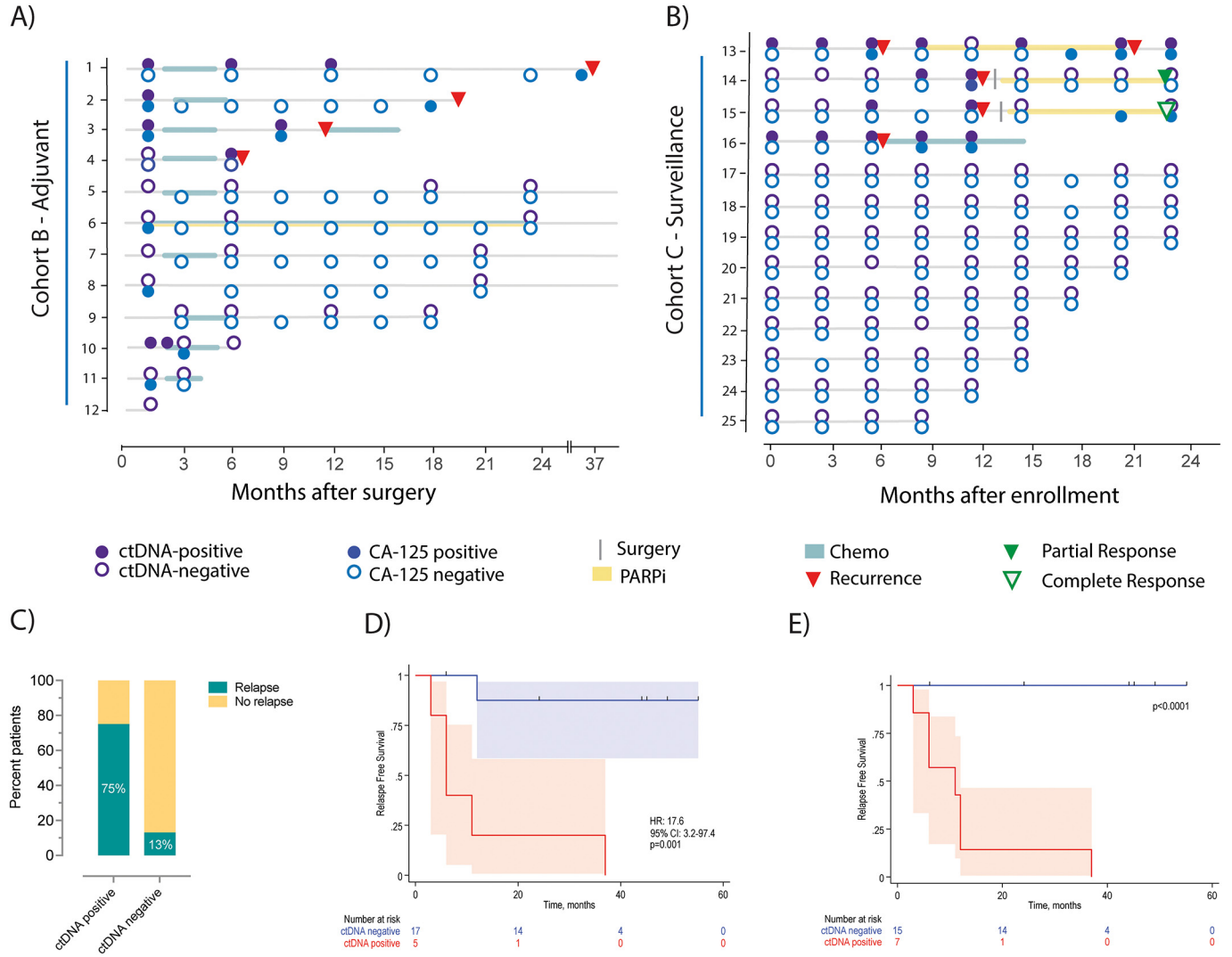


Fig. 3. A, B) Patient overview plot representing clinical follow-up for cohort B (adjuvant setting) and C (surveillance setting) Plasma samples were collected serially every 3 months for evaluation of ctDNA and CA125 (patients 5–8 had a follow up of 55,44,49,45 months respectively). C-E) Association of recurrence-free survival with ctDNA. C) MRD-positive patients had a higher rate of recurrence compared to ctDNA-negative. The median follow-up for Fig. 3C in the ctDNA-negative group was 34 months (range: 3–55 months) and in the ctDNA-positive group was 14.5 months (range: 6–37 months). D, E) Kaplan-meir estimates representing the association of ctDNA status with RFS; D) at a single time point after completion of definitive treatment, E) longitudinally.

4. Discussion

4.1. Summary of main results

In this study we demonstrate that the detection of ctDNA following debulking surgery or ACT is highly prognostic and ctDNA monitoring during surveillance is feasible for early recurrence detection. We observed that both detection and higher ctDNA levels in plasma prior to surgery was associated with higher grade histology, stage, and increased likelihood of mortality, which may help identify patients at highest risk of recurrence (Fig. 2).

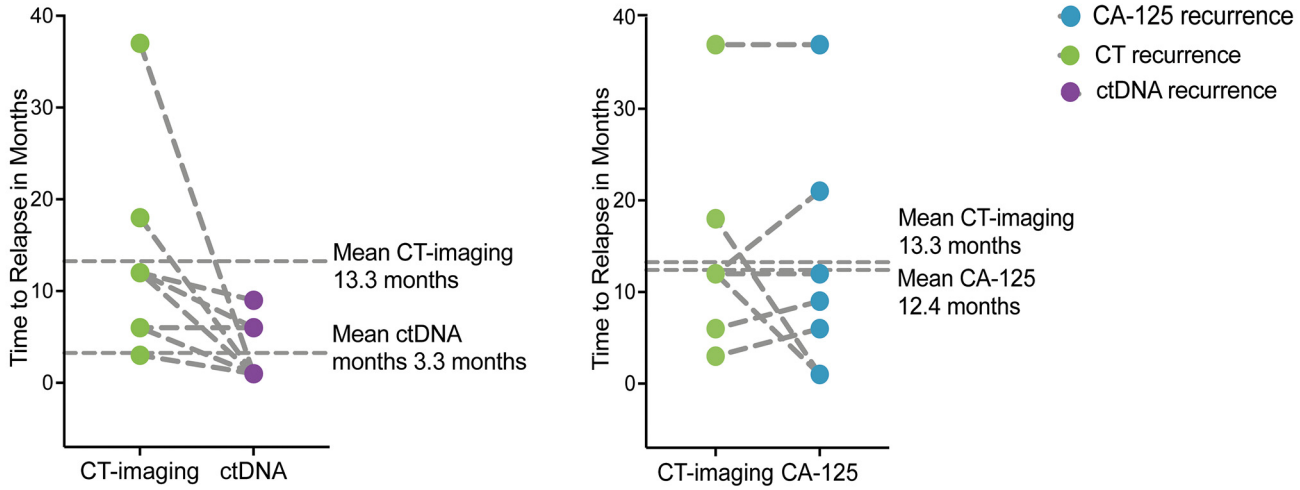
We report here that the majority of patients (75%, 3/4) with detectable ctDNA after resection experienced disease recurrence compared with only 13% (1/8) of MRD-negative patients, although this association was not statistically significant. Larger sample set is needed to formally evaluate the relationship between ctDNA detection at this timepoint and RFS. Presence of ctDNA in patient plasma during surveillance (either at a single time point or longitudinally) is significantly associated with markedly reduced RFS (single time point: HR = 17.6, 95% CI: 3.2–97.4, $p = 0.001$ and longitudinal: $p = 0.0001$, respectively) (Fig. 3D, E). ctDNA status after definitive therapy remained a significant prognostic factor when adjusting for CA-125 status, histology, or stage. Additionally, sensitivity and specificity of ctDNA monitoring for relapse prediction were observed in all patients (100%) with longitudinal monitoring ($p < 0.0001$) (Fig. 3A, B). Due to the limited sample size, we were not able to perform a more comprehensive analysis including multiple risk factors; however, our observations provide justification that further investigation is warranted and suggest that ctDNA status may add important insights to a patient's disease status and clinical outcomes.

On comparing CA-125 elevation and ctDNA detection for recurrence monitoring, we found that CA-125 was inferior in predicting recurrence when assessed at a single time point after definitive treatment ($p = 0.113$ vs. $p = 0.001$) and longitudinally ($p = 0.056$ vs. $p < 0.0001$) (Fig. 4A). ctDNA detection also preceded clinical relapse, on average 10 months ahead of radiological findings, compared to ~1 month when assessed by CA-125 status (Fig. 4B). Thus, monitoring ctDNA post-surgery and every 3 months during surveillance allowed detection of MRD with higher sensitivity than CA-125 screening and ahead of radiologic findings.

A)

	ctDNA				CA-125			
	N	Hazard Ratio	95%CI	p value	N	Hazard Ratio	95%CI	p value
Post Surgery single time point	12	7.34	0.75-72.3	0.087	6	0.45	0.06-3.4	0.439
Surveillance single time point	22	17.6	2.6-89	**0.001**	21	6.2	0.6-60	0.113
Surveillance serially	22			**<0.0001**	21	4.3	0.96-19.5	0.056

B)



C)

Univariate	Hazard Ratio	95%CI	p value
ctDNA status	13.4	2.4-75.2	**0.003**
CA-125 status	1.8	0.2-15.2	0.582
Histology	1.3	0.3-6.5	0.748
Stage	7	1.3-36.5	**0.021**
Bivariate	**Adjusted HR**	**95%CI**	**p value**
ctDNA status CA-125 status	13 1.15	2.2-77.5 0.1-12.2	**0.005** 0.907
ctDNA status Histology	13.8 0.8	2.4-80.2 0.1-4.5	**0.003** 0.791
ctDNA status Stage	14.5 19	1.3-156.2 1.5-236.1	**0.028** **0.022**

Fig. 4. Association of recurrence-free survival (RFS) with ctDNA and CA-125 and lead time analysis relative to radiological imaging: A) Results of univariate Cox regression analysis for ctDNA and CA125 status at different time points. B) Comparison of time to recurrence based on ctDNA ($n = 8$ patients) and CA-125 ($n = 7$ patients) vs. radiological imaging. Y-axis represents time to recurrence in months from the first ctDNA detection to positive imaging for each patient with recurrence, regardless of adjuvant treatment status. C) Results of univariate and bivariate Cox regression analysis for clinicopathological factors including ctDNA and CA125 status at single time point after completion of definitive treatment.

4.2. Results in the context of published literature

Consistent with previously published studies across solid tumors [16–18], our findings demonstrate that the absence of ctDNA following primary treatment was associated with improved outcomes. Kim et al. prospectively demonstrated ctDNA to be a potential tumor-specific biomarker for treatment response monitoring in HGSOC. Interestingly, the majority of studies to date have focused on analyzing the most common somatic mutations characteristic of EOC, which includes the *TP53* [19–21], *PTEN* [21], *PIK3CA* [21], and *BRCA1/2* [22,23] genes. We observed that exclusively targeting these and other driver variants such as *BRAF* and *ARID1A/B* (Supplementary Table 1) would have resulted in reduced sensitivity for MRD detection when analyzed longitudinally i.e., 40% vs. 100% for a tumor-informed mPCR-NGS-based ctDNA assay

(Supplementary Fig. 1). Thus, the assessment of a single mutation or a static panel of variants may not accurately reflect the residual disease level, while a personalized, tumor-informed ctDNA assay focused on the clonal variants may allow for higher precision when identifying the presence of disease at the molecular level. Finally, with respect to CA-125, our findings are also consistent with other studies demonstrating the poor sensitivity and specificity of CA-125 for recurrence detection. These findings illustrate the advantage of using ctDNA as a predictive biomarker for early recurrence detection.

Assessment of ctDNA treatment response following adjuvant therapy was prognostic. Of 5 patients who were MRD-positive after ACT, all recurred (within 4 weeks = 1; < 6 months = 1; > 6 months = 3). Whereas only 2 of 17 patients who were ctDNA-negative after ACT recurred, both >12 months from last platinum therapy. These results highlight the potential utility of ctDNA analysis to monitor patient treatment response and make informed decisions about treatment efficacy ahead of radiological assessment.

4.3. Strengths and weaknesses

The strength of the study is the use of prospective samples, serially collected at scheduled intervals to demonstrate the potential of ctDNA monitoring to identify recurrence and evaluate treatment effectiveness in patients with EOC. Compared to CA-125, ctDNA analysis was found to be more reliable and consistent at assessing treatment response and offers a significant lead time in identifying clinical recurrence with respect to radiological imaging. Some of the limitations in this study include the modest sample size, a heterogenous study population, and loss of follow-up for some patients.

4.4. Implications for practice and future research

Historically, early recurrence detection using either second-look laparotomy or CA-125 did not result in improved survival [24,25]. However, the evolution of maintenance strategies and newer therapies, combined with a more sensitive and specific MRD technologies such as Signatera, could provide an opportunity for early intervention, leading to a survival advantage as observed in other indications [17,26]. Longitudinal monitoring of ctDNA in the adjuvant and surveillance settings, with a focus on MRD detection and recurrence prediction, can aid in making timely decisions for personalized disease management, thereby improving a patient's chances of survival as well as improving their quality-of-life.

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Data availability

The supporting data on driver variants detected in whole-exome sequencing (WES) results that were included in the static panel have been made available in Supplementary Table 1. Clinical data and ctDNA results (mean tumor molecules/ml of plasma) at each time point and WES results can be made available upon a reasonable request.

Author contributions

Conceptualization: J.Y.H., J.C., A.A., M.B., J.M.F., P.B., and S.S.; Resources: J.Y.H., R.M.V., J.C., W.P., K.S-M., G.P., A.R., M.M.[1], C.S.J., J.M.F., T.D., R.E., and M.B.; Data curation:

W.P., M.B., T.D., C.J.S., R.E., G.P., S.S., R.M.V., M.M. [1], and A.R.; Software, Visualization: E.K., V.A., and H-T.W.; Formal analysis: E.K., S.S., S.D., H-T. W., V.A., M.M.[2], B.Z., H.S., A.E. and A.A.; Supervision: K.S-M., J.Y.H., J.C., and J.M.F.; Validation: S.D., H.S., and B.Z.; Investigation: W.P., M.B., J.M.F.,J.Y.H., and J.C.; Methodology: E.K., S.S., S.D., H-T.W., V.A., M.M.[2], A.E., B.Z., H.S., and A.A. Wrote original draft: E.K., M.M.[2] G.V.G., J.Y.H., and J.C. Project administration: S.S, T.D., R.M.V., and M.M.[1], J.Y.H. and J.C. contributed equally. All authors critically reviewed the manuscript and agreed to submit for publication.

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Conflict of interest

None.

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References

[1] C.A. Doubeni, A.R. Doubeni, A.E. Myers, Diagnosis and management of ovarian cancer, Am. Fam. Physician 93 (11) (Jun 1, 2016) 937–944, [Online]. Available https://www.ncbi.nlm.nih.gov/pubmed/27281838.

[2] R.L. Siegel, K.D. Miller, H.E. Fuchs, A. Jemal, Cancer statistics, 2022, CA Cancer J. Clin. 72 (1) (Jan 2022) 7–33, https://doi.org/10.3322/caac.21708.

[3] B.A. Goff, et al., "Development of an ovarian cancer symptom index: possibilities for earlier detection," (in eng), Cancer 109 (2) (Jan 15, 2007) 221–227, https://doi.org/10.1002/cncr.22371.

[4] National Comprehensive Cancer Network (NCCN), NCCN Clinical Practice Guidelines in Oncology: Ovarian Cancer Including Fallopian Tube Cancer and Primary Peritoneal Cancer Version 1.2022 [Online], Available: https://www.nccn.org/professionals/physician_gls/pdf/ovarian.pdf.

[5] T. Damak, R. Chargui, J. Ben Hassouna, M. Hechiche, K. Rahal, "Results of second-look laparotomy in advanced ovarian cancer: one single center experience," (in eng), ISRN Obstet. Gynecol. 2012 (2012), 849518, https://doi.org/10.5402/2012/849518.

[6] I. Vergote, et al., "Overall survival results of AGO-OVAR16: a phase 3 study of maintenance pazopanib versus placebo in women who have not progressed after first-line chemotherapy for advanced ovarian cancer," (in eng), Gynecol. Oncol. 155 (2) (Nov 2019) 186–191, https://doi.org/10.1016/j.ygyno.2019.08.024.

[7] T. Evans, U. Matulonis, "PARP inhibitors in ovarian cancer: evidence, experience and clinical potential," (in eng), Ther. Adv. Med. Oncol. 9 (4) (Apr 2017) 253–267, https://doi.org/10.1177/1758834016687254.

[8] K. Moore, et al., "Maintenance olaparib in patients with newly diagnosed advanced ovarian cancer," (in eng), N. Engl. J. Med. 379 (26) (Dec 27, 2018) 2495–2505, https://doi.org/10.1056/NEJMoa1810858.

[9] K.M. Esselen, et al., "Use of CA-125 tests and computed tomographic scans for surveillance in ovarian cancer," (in eng), JAMA Oncol. 2 (11) (Nov 1, 2016) 1427–1433, https://doi.org/10.1001/jamaoncol.2016.1842.

[10] A. Gadducci, S. Cosio, "Surveillance of patients after initial treatment of ovarian cancer," (in eng), Crit. Rev. Oncol. Hematol. 71 (1) (Jul 2009) 43–52, https://doi.org/10.1016/j.critrevonc.2008.12.008.

[11] A. Prat, et al., "Risk of recurrence during follow-up for optimally treated advanced epithelial ovarian cancer (EOC) with a low-level increase of serum CA-125 levels," (in eng), Ann. Oncol. 20 (2) (Feb 2009) 294–297, https://doi.org/10.1093/annonc/mdn601.

[12] G.J. Rustin, A.E. Nelstrop, M.K. Tuxen, H.E. Lambert, "Defining progression of ovarian carcinoma during follow-up according to CA 125: a North Thames ovary group study," (in eng), Ann. Oncol. 7 (4) (Apr 1996) 361–364, https://doi.org/10.1093/oxfordjournals.annonc.a010602.

[13] A.P. Vaidya, J.P. Curtin, "the follow-up of ovarian cancer," (in eng), Semin. Oncol. 30 (3) (Jun 2003) 401–412, https://doi.org/10.1016/s0093-7754(03)00100-3.

[14] P. Bhosale, A. Kamat, P.J. Eifel, Chapter 26 - ovarian cancer, in: P.M. Silverman (Ed.), Oncologic Imaging: A Multidisciplinary Approach, W.B. Saunders, Philadelphia 2012, pp. 455–478.

[15] L. Manganaro, et al., Imaging strategy in recurrent ovarian cancer: a practical review, Abdom. Radiol. (NY) 44 (3) (Mar 2019) 1091–1102, https://doi.org/10.1007/s00261-018-1677-y.

[16] R.C. Coombes, et al., Personalized detection of circulating tumor dna antedates breast cancer metastatic recurrence, Clin. Cancer Res. 25 (14) (Jul 15, 2019) 4255–4263, https://doi.org/10.1158/1078-0432.CCR-18-3663.

[17] T. Powles, et al., ctDNA guiding adjuvant immunotherapy in urothelial carcinoma, Nature 595 (7867) (Jul 2021) 432–437, https://doi.org/10.1038/s41586-021-03642-9.

[18] T. Reinert, et al., "Analysis of plasma cell-free DNA by ultradeep sequencing in patients with stages I to III colorectal cancer," (in eng), JAMA Oncol. 5 (8) (Aug 1, 2019) 1124–1131, https://doi.org/10.1001/jamaoncol.2019.0528.

[19] Y.M. Kim, S.W. Lee, Y.J. Lee, H.Y. Lee, J.E. Lee, E.K. Choi, "Prospective study of the efficacy and utility of TP53 mutations in circulating tumor DNA as a non-invasive biomarker of treatment response monitoring in patients with high-grade serous ovarian carcinoma," (in eng), J. Gynecol. Oncol. 30 (3) (May 2019), e32, https://doi.org/10.3802/jgo.2019.30.e32.

[20] C.A. Parkinson, et al., "Exploratory analysis of TP53 mutations in circulating tumour DNA as biomarkers of treatment response for patients with relapsed high-grade serous ovarian carcinoma: a retrospective study," (in eng), PLoS Med. 13 (12) (Dec 2016), e1002198, https://doi.org/10.1371/journal.pmed.1002198.

[21] E. Pereira, et al., "Personalized circulating tumor DNA biomarkers dynamically predict treatment response and survival in gynecologic cancers," (in eng), PLoS One 10 (12) (2015), e0145754, https://doi.org/10.1371/journal.pone.0145754.

[22] K.K. Lin, et al., "BRCA reversion mutations in circulating tumor DNA predict primary and acquired resistance to the PARP inhibitor Rucaparib in high-grade ovarian carcinoma," (in eng), Cancer Discov. 9 (2) (Feb 2019) 210–219, https://doi.org/10.1158/2159-8290.CD-18-0715.

[23] M. Ratajska, et al., "Detection of BRCA1/2 mutations in circulating tumor DNA from patients with ovarian cancer," (in eng), Oncotarget 8 (60) (Nov 24, 2017) 101325–101332, https://doi.org/10.18632/oncotarget.20722.

[24] J.S. Berek, N.F. Hacker, L.D. Lagasse, T. Poth, B. Resnick, R.K. Nieberg, Second-look laparotomy in stage III epithelial ovarian cancer: clinical variables associated with disease status, Obstet. Gynecol. 64 (2) (Aug 1984) 207–212, [Online]. Available https://www.ncbi.nlm.nih.gov/pubmed/6539884.

[25] S.K. Chambers, J.T. Chambers, E.I. Kohorn, R. Lawrence, P.E. Schwartz, Evaluation of the role of second-look surgery in ovarian cancer, Obstet. Gynecol. 72 (3 Pt 1) (Sep 1988) 404–408, [Online]. Available https://www.ncbi.nlm.nih.gov/pubmed/3405557.

[26] M. Kotaka, et al., Association of circulating tumor DNA dynamics with clinical outcomes in the adjuvant setting for patients with colorectal cancer from an observational GALAXY study in CIRCULATE-Japan, J. Clin. Oncol. 40 (4_suppl) (2022) 9, https://doi.org/10.1200/JCO.2022.40.4_suppl.009.