Received: 26 August 2022 | Revised: 4 January 2023 | Accepted: 11 January 2023

DOI: 10.1002/cncr.34724

ORIGINAL ARTICLE

Clinical implications of tumor-based next-generation sequencing in high-grade epithelial ovarian cancer

Katherine I. Foster MD | Kenna R. M. Shaw PhD | Jeff Jin PhD | Shannon N. Westin MD | Timothy A. Yap MBBS, PhD | Deanna M. Glassman MD | Amir A. Jazaeri MD | Jose A. Rauh-Hain MD | Sanghoon Lee PhD | Bryan M. Fellman MS | Zhenlin Ju PhD | Yuexin Liu PhD | Nicole D. Fleming MD | Anil K. Sood MD

The University of Texas MD Anderson Cancer Center, Houston, Texas, USA

Correspondence
Anil K. Sood, Department of Gynecologic Oncology & Reproductive Medicine, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd., Unit 1362, Houston, TX 77030-4009, USA.
Email: asood@mdanderson.org

Funding information
National Institutes of Health, Grant/Award Numbers: CA217685, CA209904, CA101642, CA016672; The University of Texas MD Anderson Cancer Center Ovarian Cancer SPORE; American Cancer Society; Ovarian Cancer Research Alliance; Dunwoody Fund; Khalifa Bin Zayed Al Nahyan Foundation; Gordon Fund; Frank McGraw Memorial Chair in Cancer Research; The University of Texas MD Anderson Ovarian Cancer Moon Shot

Abstract

Background: Tumor-based next-generation sequencing is used inconsistently as a tool to tailor treatment of ovarian cancer, yet beyond detection of somatic *BRCA1* and *BRCA2* mutations, the clinical benefit is not well established. This study aimed to assess the clinical relevance of tumor-based next-generation sequencing (tbNGS) in patients with ovarian cancer.

Methods: This retrospective study included patients with high-grade epithelial ovarian carcinoma. tbNGS results were identified in the electronic medical record using optical character recognition and natural language processing. Genetic, clinical, and demographic information was collected. Progression-free survival (PFS) and overall survival were calculated and compared using log-rank tests. Multivariate Cox regression and clustering analyses were used to identify patterns of genetic alterations associated with survival.

Results: Of 1092 patients in the described population, 409 (37.5%) had tbNGS results. Nearly all (96.1% [393/409]) had one or more genetic alterations. In 25.9% (106/409) of patients, an alteration that aligned with a targeted treatment was identified, and in an additional 48.7% (199/409), tbNGS results suggested eligibility for an investigational agent or clinical trial. The most frequent alterations were *TP53*, *PIK3CA*, and *NF1* mutations, and *CCNE1* amplification. Together, *BRCA1* and *BRCA2* mutations were associated with longer PFS (hazard ratio [HR], 0.62; 95% confidence interval [CI], 0.42–0.92; $p = .02$), whereas *AKT2* amplification was associated with shorter PFS (HR, 3.86; 95% CI, 1.002–14.88; $p < .05$). Multivariate Cox regression and clustering analyses identified several combinations of genetic alterations that corresponded to outcomes in patients with high-grade serous carcinoma.

Conclusions: tbNGS often yields clinically relevant information. Detailed analysis of population-level tumor genomics may help to identify therapeutic targets and guide development of clinical decision support tools.

The first three authors contributed equally to this article.

Plain Language Summary

- Although more and more patients with ovarian cancer are undergoing tumor-based next-generation sequencing to identify genetic mutations in their tumors, the benefits of such testing are not well established.
- In a group of over 400 patients with ovarian cancer who underwent tumor-based next-generation sequencing in the course of their treatment, nearly all patients had one or more genetic alterations detected, and one out of four patients had a mutation that qualified them for a personalized treatment option.

KEYWORDS

genetic testing, high-throughput nucleotide sequencing, mutation, natural language processing, ovarian epithelial carcinoma, ovarian neoplasms, retrospective studies

INTRODUCTION

Ovarian cancer afflicts approximately 20,000 women per year in the United States.[1] Epithelial ovarian carcinomas, which represent the vast majority of ovarian cancers, are recognized as a heterogeneous group.[2] Different histologic subtypes of epithelial ovarian cancer have distinct pathologic and genetic features. Genetic heterogeneity exists even among patients with the same subtype, as well as spatially and temporally within the same patient.

Despite our growing understanding of the heterogeneity in epithelial ovarian cancer, upfront treatment remains nonspecific, and there have been few true advancements in patient outcomes. Regardless of histologic subtype or molecular profile, cytotoxic chemotherapy and surgical debulking remain the standard of care for primary treatment of epithelial ovarian cancer.[3] Poly(adenosine diphosphate ribose) polymerase (PARP) inhibitors, indicated for patients with *BRCA1* or *BRCA2* mutations and other forms of homologous recombination deficiency, were the first molecularly targeted treatments for ovarian cancer.[4] There is a pressing need for additional effective targeted therapies to improve outcomes of patients with this disease. Developing such therapies will require a comprehensive and nuanced understanding of the landscape of genomic alterations in ovarian cancer. Such genetic analysis was traditionally done using Sanger sequencing to analyze a particular gene of interest; today, next-generation sequencing (NGS) allows for simultaneous analysis of thousands of genes with a clinically feasible cost and turnaround time.[5]

Targeted NGS, using panels designed to detect point mutations, small insertions and/or deletions, and occasionally copy number variants (CNVs) and gene fusions in a defined set of relevant genes, is the method of choice for detection of somatic variants in tumor tissue.[6] Tumor-based NGS (tbNGS) can serve as a tool to tailor treatment strategy, yet beyond detection of somatic *BRCA1* and *BRCA2* mutations, the clinical benefit of tbNGS in ovarian cancer is largely unknown. We therefore aimed to assess the clinical relevance of tbNGS in a large cohort of patients with high-grade epithelial ovarian cancer (HGEOC).

MATERIALS AND METHODS

This retrospective cohort study was performed at The University of Texas MD Anderson Cancer Center (MDACC). The study population comprised patients with suspected advanced-stage ovarian, fallopian tube, or primary peritoneal cancer who were enrolled in the Ovarian Cancer Moon Shot program between April 1, 2013 and September 27, 2021 and were diagnosed with HGEOC. Clinical information for this population was prospectively collected and stored on the secure Research Electronic Data Capture (REDCap) platform.[7] tbNGS data were sought by querying the MDACC Khalifa Institute for Personalized Cancer Therapy (IPCT) database for molecular testing results and by scanning electronic medical records (both internal notes and external scanned documents) for genetic reports using optical character recognition (OCR) and natural language processing (NLP) technologies. Non-tbNGS genetic reports (i.e., germline testing) were excluded.

The following data were collected: panel name, date of testing, genes altered, and alteration details. Genetic alterations included any point mutations, insertions, deletions, rearrangements, duplications, and CNVs detected by the panels used. A genetic alteration was considered useful for clinical decision-making if it qualified the patient for on-label use of a United States Food and Drug Administration (FDA)-approved targeted therapy, literature-based off-label use of an FDA-approved or investigational targeted therapy, or biomarker-matched clinical trial enrollment according to the MDACC Precision Oncology Decision Support System (PODSS). Mutations and CNVs were defined as indicated by tbNGS reports; variants of undetermined significance were excluded. Wild-type (WT) genotype was assumed in patients who had undergone testing by a panel that included a particular locus but did not indicate a mutation. The scope of each panel was identified by reviewing representative results reports. Clinicodemographic data were extracted from the REDCap database on November 11, 2021.

Data were summarized by descriptive statistics. Characteristics were compared between patients with and without tbNGS results by *t*-tests, χ^2 tests, or Fisher's exact tests using GraphPad Prism

v9.0.0. Progression-free survival (PFS) was calculated from the date of first treatment to the date of first recurrence or progression; if recurrence or progression had not occurred, PFS was censored at the date of last clinic visit. Overall survival (OS) was calculated from the date of first treatment to the date of death; if death had not occurred, OS was censored at the date of last contact. The most frequent mutations and CNVs were compared by patient PFS and OS using log-rank tests in GraphPad Prism v9.0.0.

To minimize known heterogeneity between subtypes and concentrate on the most clinically relevant cohort, we performed focused analyses on patients with high-grade serous and endometrioid histologies. We selected genes that were assessed in at least 65% (median percentage) of patients and altered in at least three patients (the third quantile) to avoid genes that were mutated at low frequency. Individual alterations were evaluated for associations with survival by univariate Cox regression analysis. Multivariate Cox regression modeling and clustering analyses were used to identify combinations of alterations that were significantly associated with PFS and OS (log-rank test p value <.05). In the multivariate Cox regression analysis, we used a p value of <.1 to select the top gene predictors that favored better (hazard ratio [HR], <1) or worse prognosis (HR, >1). For clustering analysis, we applied "Gower distance" as a dissimilarity metric and "complete" as a clustering method. The Benjamini-Hochberg procedure was used to adjust for false discovery rate wherever multiple comparisons were performed. For these combination analyses, tumors were defined as mutated if any one or more of the stated genes had a point mutation or CNV and WT if all of the genes were WT; patients with missing data for any of the relevant genes were excluded. Henceforth, mutated gene sets defined in this way will be notated with colons separating the gene names (e.g., *NOTCH3:MET:PIK3R1:AKT2:PIK3CA*).

Co- and contra-mutations were identified by first performing Fisher's exact tests to identify significantly associated mutations ($p < .05$) and frequency tests to identify genes mutated in at least 50% of cases in which the other gene was also mutated (for co-mutations) or WT (for contra-mutations). Genes meeting these criteria were considered co-mutated with another gene if they were mutated more than four times as often by frequency when the other gene was mutated, and contra-mutated if they were mutated more than four times as often when the other gene was WT.

Bias was minimized by collecting and analyzing genetic results before collection of clinical data, effectively blinding the researchers to clinical outcomes. The use of OCR and NLP to scan all eligible patient records prevented our analysis from being biased toward those already enrolled in the IPCT database. Finally, utility for clinical decision-making was defined in a patient-agnostic manner. No a priori power analysis was performed as sample size was limited to the number of patients having undergone tbNGS. This study was approved by MDACC's institutional review board (PA16-1010 and PA14-0353), and all patients provided written informed consent.

RESULTS

Most patients who underwent tbNGS received clinically useful results

We identified 1392 patients with suspected advanced-stage ovarian, fallopian tube, or primary peritoneal cancer in the Ovarian Cancer Moon Shot database. Of these, 1092 were ultimately diagnosed with HGEOC and were thus included in our study (Figure S1). A total of 409 of these patients (37.5%) had undergone tbNGS. Clinical and demographic information for this population is presented in Tables S1-S3. Select demographic and clinical characteristics are compared between patients with and without tbNGS testing in Table 1. Patients with tbNGS results had a slightly younger median age (63 vs. 65 y, $p = .005$) and were more likely to have been diagnosed with stage IV disease (46.7% [191 of 409] vs. 40.0% [273 of 683], $p = .03$), have an Eastern Cooperative Oncology Group performance status of 0 (57.2% [234 of 409] vs. 49.3% [337 of 683], $p = .01$), and have undergone neoadjuvant chemotherapy (NACT) as their primary treatment (78.0% [319 of 409] vs. 46.7% [319 of 683], $p < .001$).

A summary of the tbNGS results is presented in Figure 1. Among the 409 tbNGS results, genetic alterations (i.e., mutation or CNV) were identified in 393 patients. Nearly all (95.8% [392 of 409]) had at least one mutation and approximately 20% (75 of 409) had a CNV. Each patient had a mean ($\pm$ standard deviation) of 2.12 $\pm$ 2.30 mutations and 0.48 $\pm$ 1.96 CNVs. Most patients (74.6% [305 of 409]) had an alteration that was considered useful for clinical decision-making, defined as one that suggested efficacy of a targeted therapy or conferred eligibility for a biomarker-matched trial according to the PODSS. Seven (1.7%) of the patients were eligible for on-label use of an FDA-approved treatment, in all cases PARP inhibitors for deleterious *BRCA1* or *BRCA2* mutations. A total of 24.7% (101 of 409) of patients had genetic alterations that were suited for evidence-based, off-label use of an available, FDA-approved agent, such as PI3K or mTOR inhibitors for activating *PIK3CA* mutations or MEK inhibitors for activating *KRAS* or *NRAS* mutations. Eighty-six patients (21.0%) had alterations suggesting efficacy of a targeted investigational treatment, and 260 patients (63.6%) met criteria for a biomarker-matched clinical trial. Details of all detected mutations and CNVs considered to be useful for clinical decision-making are presented in Tables S4 and S5.

tbNGS panels have expanded over time

The 10 tbNGS assays used in this population included six institutional and four commercial panels. The scope of these panels varied widely, ranging from 35 to 648 genes assessed. Details of the panels used are provided in Table S6. In general, the breadth of the panels used has expanded over time, from an average of 75 genes assessed for mutations in 2015 to 260 in 2021. Not all of these assays detect CNVs, although most (7 of 10) do. The most commonly used test

TABLE 1 Comparison of demographic and clinical information of patients with and without tbNGS.

Characteristic	With tbNGS (*N* = 409), No. (%)	Without tbNGS (*N* = 683), No. (%)	*p*
Age (year)[a]	63 (55–70)	65 (56–73)	.005
BMI (kg/m^2)[a]	26.5 (22.9–30.6)	27.0 (23.4–31.2)	.10
Race[b]			.39
American Indian or Alaskan Native	1 (0.2)	3 (0.4)	
Asian	23 (5.6)	35 (5.1)	
Black	37 (9.0)	40 (5.9)	
Pacific Islander	0 (0.0)	1 (0.1)	
White	327 (80.0)	561 (82.1)	
Other	9 (2.2)	24 (3.5)	
Unknown	12 (2.9)	19 (2.8)	
Ethnicity[b]			.73
Hispanic or Latino	50 (12.2)	95 (13.9)	
Not Hispanic or Latino	354 (86.6)	580 (84.9)	
Unknown	5 (1.2)	8 (1.2)	
Primary disease site			.09
Ovary	298 (72.9)	537 (78.6)	
Peritoneum	84 (20.5)	109 (16.0)	
Fallopian tube	27 (6.6)	37 (5.4)	
Histology			.56
Serous	364 (89.0)	608 (89.0)	
Clear cell	17 (4.2)	16 (2.3)	
Endometrioid	9 (2.2)	19 (2.8)	
Mucinous	1 (0.2)	3 (0.4)	
Mixed epithelial	11 (2.7)	23 (3.4)	
Adenocarcinoma NOS	7 (1.7)	14 (2.0)	
Stage[c]			<.001
I	0 (0.0)	19 (2.8)	
II	12 (2.9)	2 (0.3)	
III	206 (50.4)	389 (57.0)	
IV	191 (46.7)	273 (40.0)	
ECOG performance status			.025
0	234 (57.2)	337 (49.3)	
1	120 (29.3)	200 (29.3)	
2	29 (7.1)	67 (9.8)	
3	11 (2.7)	39 (5.7)	
4	2 (0.5)	4 (0.6)	
Unknown	13 (3.2)	36 (5.3)	
Primary treatment			<.001
Tumor reductive surgery	90 (22.0)	361 (52.9)	
Neoadjuvant chemotherapy	319 (78.0)	319 (46.7)	
Unknown	0 (0.0)	3 (0.4)	

Abbreviations: BMI, body mass index; ECOG, Eastern Cooperative Oncology Group; NOS, not otherwise specified; tbNGS, tumor-based next-generation sequencing.

[a]Median (interquartile range).

[b]Patient-reported.

[c]American Joint Committee on Cancer (AJCC) 8th ed.

was the institutional STGA-DNA 2018, which scans 134 genes for mutations and 47 for CNVs.

tbNGS was often employed at the presumed time of recurrence

A minority of patients (17.8% [73 of 409]) completed testing within 180 days of diagnosis, the approximate time frame of primary treatment. The median time of sequencing was 14.5 months after diagnosis. Many patients (32.5% [133 of 409]) had tbNGS done more than 2 years after their initial diagnosis (Figure S2). We observed a slow shift toward earlier testing over the study period—the mean (± standard deviation) interval from diagnosis to testing was 411 ± 178 days for patients diagnosed in 2013, compared to 312 ± 170 days for those diagnosed in 2019. Among all patients diagnosed with HGEOC between 2013 and 2020, there was a steady increase in the proportion of patients undergoing tbNGS within 180 days of diagnosis (Figure S3). Because tbNGS is often done on archival specimens, it should be noted that the timing of tbNGS testing completion is not necessarily reflective of the timing of specimen collection; 60.1% (246 of 409) of tbNGS testing in this cohort was performed on samples collected within 180 days of diagnosis.

TP53 mutations, *PIK3CA* mutations, and *CCNE1* amplifications were the most common somatic alterations

The most frequently mutated genes were *TP53* (86.6% [354 of 409]), *PIK3CA* (8.1% [33 of 409]), *NF1* (6.4% [22 of 346]), *KRAS* (4.6% [19 of 409]), *ARID1A* (6.5% [18 of 278]), *BRCA2* (4.1% [14 of 343]), *BRCA1* (3.2% [11 of 343]), *CDK12* (3.6% [10 of 278]), *PPP2R1A* (2.9% [10 of 345]), *NOTCH3* (3.3% [9 of 270]), and *NOTCH1* (2.0% [8 of 409]) (Table 2). Notably, many tbNGS assays are designed to intentionally exclude germline mutations; therefore, when interpreting standalone

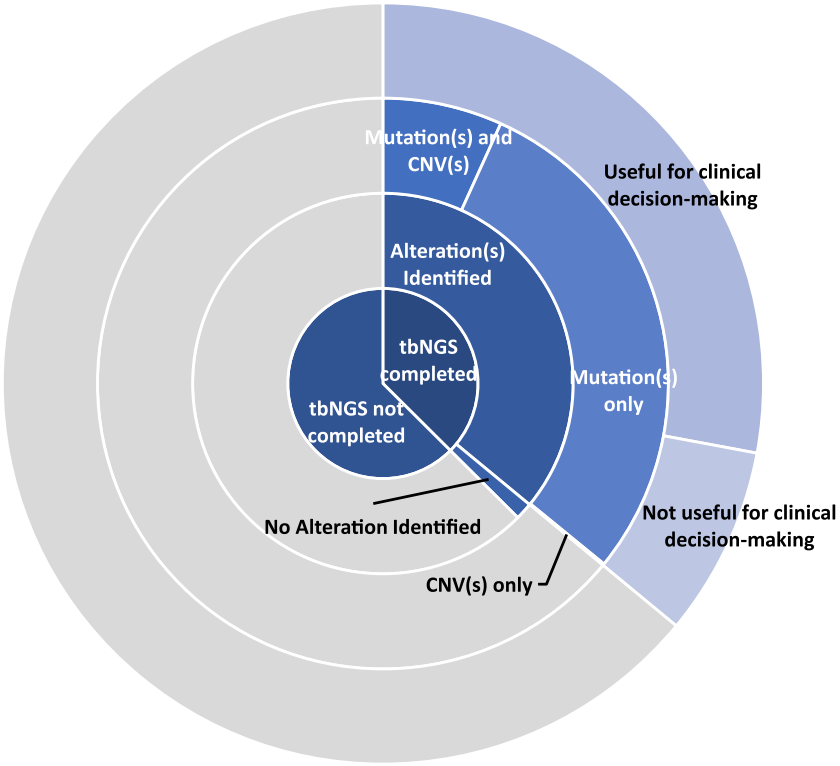

FIGURE 1 tbNGS results summary. CNV indicates copy number variant; tbNGS, tumor-based next-generation sequencing.

TABLE 2 Most frequently mutated genes.

	Patients with mutation			Patients with wild-type			HR (95% CI)		*p*	
Gene	No. (%)	PFS[a] (mo)	OS[a] (mo)	No. (%)	PFS[a] (mo)	OS[a] (mo)	PFS	OS	PFS	OS
ARID1A	18 (6.5)	18.1	67.4	260 (93.5)	15.2	47.1	0.96 (0.56–1.65)	1.17 (0.48–2.81)	.89	.73
BRCA1	11 (3.2)	22.3	44.4	332 (96.8)	14.3	46.3	0.65 (0.36–1.17)	0.70 (0.31–1.58)	.15	.39
BRCA2	14 (4.1)	16.1	51.9	329 (95.9)	14.1	44.4	0.67 (0.41–1.10)	0.73 (0.36–1.44)	.11	.36
CDK12	10 (3.6)	15.3	62.5	268 (96.4)	15.3	46.3	1.11 (0.55–2.24)	0.57 (0.22–1.48)	.77	.25
KRAS	19 (4.6)	15.3	62.5	390 (95.4)	13.9	42.3	1.02 (0.62–1.68)	0.70 (0.40–1.22)	.93	.21
NF1	22 (6.4)	18.0	81.3	324 (93.6)	14.1	44.4	0.90 (0.56–1.44)	0.69 (0.37–1.30)	.66	.25
NOTCH1	8 (2.0)	17.9	29.7	401 (98.0)	13.9	43.9	0.99 (0.49–2.00)	1.42 (0.51–3.98)	.99	.50
NOTCH3	9 (3.3)	14.1	57.1	261 (96.7)	15.3	46.3	1.03 (0.45–2.34)	1.61 (0.48–5.35)	.95	.44
PIK3CA	33 (8.1)	14.0	50.6	376 (91.9)	13.9	42.3	1.16 (0.77–1.74)	0.86 (0.54–1.35)	.49	.50
PPP2R1A	10 (2.9)	9.4	Und.	335 (97.1)	14.4	44.4	0.77 (0.39–1.51)	0.55 (0.25–1.19)	.45	.13
TP53	354 (86.6)	14.0	41.7	55 (13.4)	11.5	51.0	0.86 (0.61–1.20)	0.99 (0.68–1.4)	.37	.97

Abbreviations: CI, confidence interval; mo, months; HR, hazard ratio; OS, overall survival; PFS, progression-free survival; Und., undefined.
[a]Median.

results, patients with germline mutations (e.g., *BRCA1* or *BRCA2*) may be erroneously assumed to have a WT genotype. The most common types of mutations were missense (73.1% [299 of 409]), frameshift (22.2% [91 of 409]), and nonsense (18.3% [75 of 409]) substitutions. All identified CNVs were amplifications. The most frequently amplified genes were *CCNE1* (7.6% [26 of 343]), *KRAS* (3.9% [13 of 337]), *MYC* (1.7% [6 of 343]), *AKT2* (1.8% [5 of 273]), and *MDM2* (1.5% [5 of 342]) (Table 3).

Aberrations in *TP53* were considered useful for clinical decision-making in 59.4% of patients (243 of 409). In all such cases, the utility for clinical decision-making was based on eligibility for a clinical trial. By nature, clinical trial enrollment criteria are a moving target and

thus the utility of alterations for clinical decision-making by this definition is not static. At the time of publication, NCT04585750 is currently enrolling patients with advanced solid malignancies with a *TP53* Y220C mutation. NCT01357161 previously recruited patients with high-grade ovarian cancer with any somatic *TP53* mutation. In general, the number of biomarker-matched clinical trials is expanding over time,[8] and thus the utility of tbNGS for identifying eligible patients is also expected to increase.

Several somatic genetic patterns were significantly associated with survival outcomes

When taken together, *BRCA1* and *BRCA2* mutations were associated with significantly better PFS than *BRCA1* and *BRCA2* WT (HR, 0.62; 95% CI, 0.42–0.92; $p = .02$) (Figure 2A). *AKT2* amplification, although affecting only five patients, was associated with shortened PFS (HR, 3.86; 95% CI, 1.002–14.9; $p = .0497$) (Figure 2B). None of the other prevalent alterations were individually associated with PFS or OS (Tables 2 and 3).

In patients with high-grade serous carcinoma ($n = 364$), multivariate Cox regression identified two gene combinations in which somatic alterations in any one or more of the genes were significantly associated with PFS. *NOTCH3:MET:PIK3R1:AKT2:PIK3CA* alterations were associated with worse PFS than WT (HR, 1.67; 95% CI, 1.22–2.28; $p = .001$) (Figure 3A), whereas *ATRX:NF2* alterations were associated with better PFS (HR, 0.48; 95% CI, 0.23–0.97; $p = .04$) (Figure 3B). Multivariate Cox modeling also found that *MET:NOTCH3:CREBBP:ATR* alterations were associated with poor OS (HR, 2.24; 95% CI, 1.30–3.85; $p = .003$) (Figure 3C).

Because of biological similarities and shared treatment algorithms, we repeated the same analysis including patients with both high-grade serous ($n = 364$) and high-grade endometrioid ($n = 9$) histologic subtypes. Similar genetic combinations were associated with poor prognosis: *NOTCH3:MET:PIK3R1:ATR* alterations were associated with worse PFS (HR, 2.07; 95% CI, 1.35–3.17; $p < .001$) (Figure S4A), and *MET:NOTCH3:CREBBP* alterations were associated with shorter OS (HR, 2.20; 95% CI, 1.22–3.95; $p = .007$) (Figure S4B). Similarly, *ATRX:NF2* alterations were associated with longer PFS (HR, 0.49; 95% CI, 0.24–1.00; $p = .046$) (Figure S4C). An additional combination, *ATM:FGFR1:CDKN2A:FGFR2:NF2:FBXW7:NRAS*, was associated with longer PFS (HR, 0.65; 95% CI, 0.43–0.9975; $p = .047$) by clustering analysis (Figure S4D).

Six genes had frequent co-mutations in high-grade serous ovarian cancers

In patients with high-grade serous carcinoma, we identified six genes with co-mutations. *CCNE1* was co-mutated with *BRCA1* or *BRCA2*

TABLE 3 Most frequently amplified genes.

	Patients with amplification			Patients with wild-type			HR (95% CI)		*p*	
Gene	No. (%)	PFS[a] (mo)	OS[a] (mo)	No. (%)	PFS[a] (mo)	OS[a] (mo)	PFS	OS	PFS	OS
AKT2	5 (1.8)	13.0	29.2	268 (98.2)	15.6	47.2	3.86 (1.00–14.88)	0.82 (0.23–2.90)	.0497	.75
CCNE1	26 (7.6)	13.7	40.8	317 (92.4)	14.5	46.3	1.01 (0.69–1.70)	1.07 (0.61–1.89)	.74	.81
KRAS	13 (3.9)	12.4	48.3	324 (96.1)	14.4	44.4	1.32 (0.62–2.80)	1.57 (0.64–3.88)	.47	.33
MDM2	5 (1.5)	20.3	49.7	337 (98.5)	14.4	45.0	1.02 (0.38–2.78)	1.14 (0.40–3.29)	.97	.80
MYC	6 (1.7)	19.3	51.9	337 (98.3)	14.4	45.0	0.70 (0.31–1.56)	1.03 (0.32–3.28)	.38	.96

Abbreviations: CI, confidence interval; HR, hazard ratio; mo, months; OS, overall survival; PFS, progression-free survival.
[a]Median.

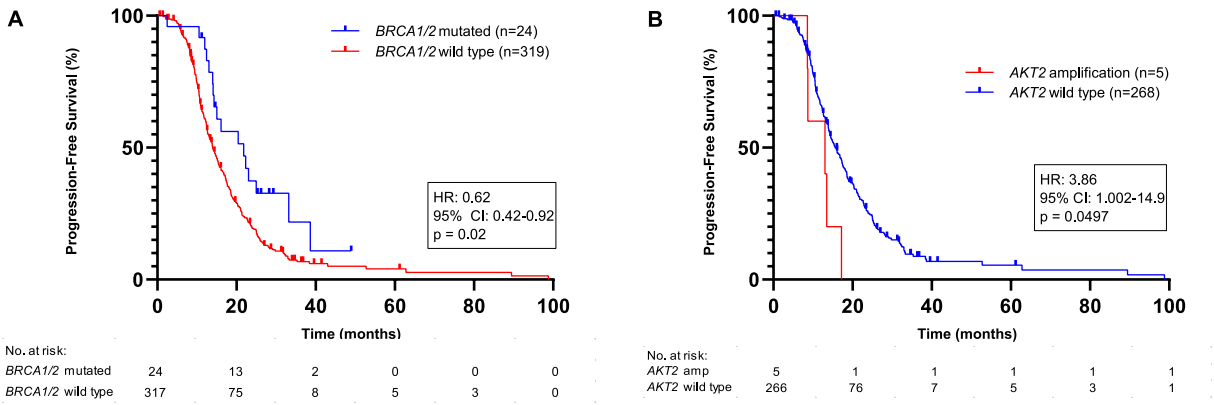

FIGURE 2 Kaplan–Meier plots of progression-free survival in patients with (A) *BRCA1/2* mutation and (B) *AKT2* amplification compared to those with WT. Amp indicates amplified; HR, hazard ratio; Mut, mutated; WT, wild-type.

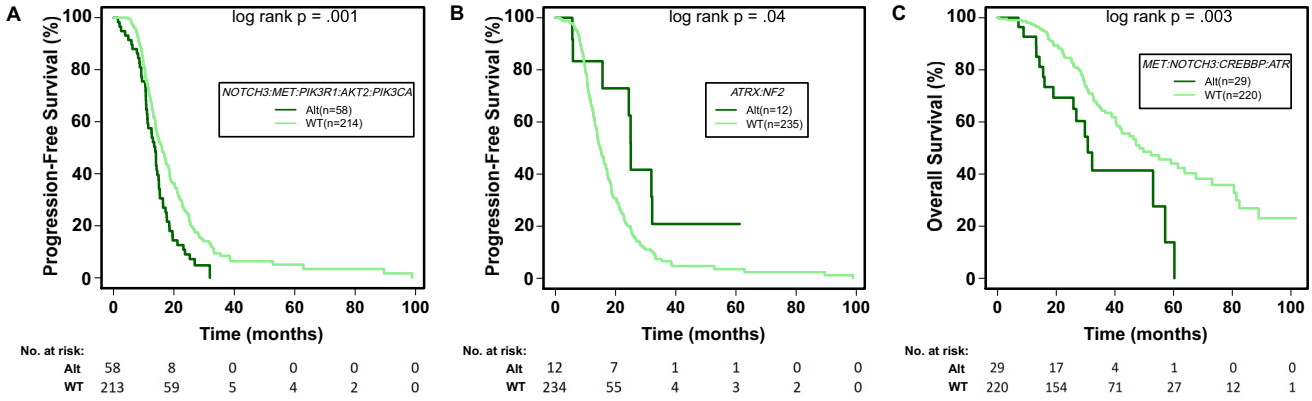

FIGURE 3 Kaplan–Meier plots of survival in patients with high-grade serous ovarian cancer with somatic alteration combinations (Alt) identified by multivariate Cox modeling compared to patients with wild-type (WT). (A) *NOTCH3:MET:PIK3R1:AKT2:PIK3CA* progression-free survival; (B) *ATRX:NF2* progression-free survival; (C) *MET:NOTCH3:CREBBP:ATR* overall survival.

(mutated in 66.7% of cases in which *CCNE1* was mutated vs. 7.3% of cases in which *CCNE1* was WT, $p = .02$). *ATRX* was co-mutated with *NF1* (50.0% vs. 7.1%, $p = .008$). *JAK3* was also co-mutated with *NF1* (66.7% vs. 7.0%, $p = .02$) and with *ATRX* (50.0% vs. 2.1%, $p = .048$). *MET* was co-mutated with *NOTCH2* (50.0% vs. 1.2%, $p = .03$) and *PPP2R1A* (50.0% vs. 1.3%, $p = .03$). *NOTCH2* and *PPP2R1A* were also co-mutated with one another (50.0% vs. 1.3%, $p = .002$). Finally, *NRAS* was co-mutated with *KRAS* (50.0% vs. 2.4%, $p = .004$) (Figure S5).

Very similar patterns were seen when we included patients with high-grade endometrioid histology. All of the co-mutations detailed above were confirmed in this population. In addition, *CCNE1* was co-mutated with *PTEN* (50.0% vs. 1.1%, $p = .03$; Figure S6). Though it did not meet statistical criteria for co-amplification, we noted a quantitative trend toward co-occurrence of *CCNE1* and *AKT2* amplifications, whereby *CCNE1* was amplified in 40.0% of cases in which *AKT2* was also amplified compared to 8.2% of cases in which *AKT2* was not amplified ($p = .06$).

DISCUSSION

At least one somatic genetic alteration was identified in almost all patients undergoing tbNGS in our patient population. One in four patients was a potential candidate for an available targeted therapy; 1.7% for on-label use and 24.7% for off-label use of an FDA-approved agent. This proportion increased to three of four patients when investigational agents and clinical trial eligibility were considered. Patients with tbNGS results were more often those whose cancers were diagnosed at an advanced stage, those who received NACT, younger patients, and those with better performance status; characteristics perhaps reflecting a need and appropriateness for second-line agents and/or trial enrollment among patients selected for tbNGS testing. This interpretation is also supported by the median time until sequencing, which corresponds to the likely time of disease recurrence.[9] As NGS techniques and targeted treatment options continue to evolve, questions remain regarding the optimal approach for tbNGS in the clinical setting. Our findings support the clinical relevance of tbNGS in the management of HGEOC, and we advocate for consideration of tbNGS as an adjunctive diagnostic tool in women with primary or recurrent ovarian cancer.

Although our study was not able to identify patients who received sequence-matched therapies nor was it powered to detect survival differences in patients enrolled in genotypically relevant clinical trials, the efficacy of FDA-approved molecularly targeted therapies (e.g., PARP inhibitors, anti-PD-1 monoclonal antibodies, and TRK inhibitors) has been established.[10–15] Furthermore, several meta-analyses have demonstrated that clinical trials employing personalized strategies have better outcomes as compared to non-personalized trials.[16–18]

Our identification of both individual somatic mutations and combinations thereof which correspond to outcomes contributes to the larger body of knowledge about HGEOC and posits these specific mutations as prognostic factors and potential biomarkers for targeted therapies. Our study reaffirmed *BRCA1* and *BRCA2* mutations as being associated with improved survival, confirming the *BRCA*-null phenotype as one worth exploiting for therapeutic benefit. Additionally, we identified *AKT2* amplification, which has previously been implicated in ovarian cancer tumorigenesis,[19] as a poor prognostic factor and thus worthy of consideration as a therapeutic target. In fact, Akt inhibitors have been studied for decades with promising preclinical results but limited benefit in trials. Akt overexpression and other aberrations in the PI3K/Akt/mTOR pathway detected by tbNGS may serve as biomarkers for further studies of Akt inhibitor therapy.

The genomic data presented here are expected to be generalizable to patients with HGEOC in the United States, who have comparable demographics and clinical characteristics.[20] However, we acknowledge that not all suggested treatment options, particularly investigational agents and clinical trials, will be practically available to every patient. This study was limited by its retrospective nature, which prevented a complete understanding of when and why testing was ordered and how the results were interpreted and applied.

Future studies will ideally be prospective. Although this study did attain a large sample size, it was restricted to a single institution. A more global understanding of tbNGS trends could be achieved by including multiple centers in future research.

tbNGS can provide important clinical information and has the potential to improve patient outcomes when results are effectively integrated into treatment planning. Additionally, description of population-level genomics in ovarian cancer may aid research efforts and guide development of clinical decision support tools.

AUTHOR CONTRIBUTIONS

Katherine I. Foster: Conceptualization, methodology, formal analysis, investigation, data curation, writing–original draft, writing–review and editing, and visualization. **Kenna R. M. Shaw:** Conceptualization, methodology, formal analysis, investigation, resources, data curation, writing–review and editing, supervision, project administration, and funding acquisition. **Jeff Jin:** Conceptualization, methodology, software, investigation, resources, data curation, and writing–review and editing. **Shannon N. Westin:** Conceptualization, methodology, resources, data curation, and writing–review and editing. **Timothy A. Yap:** Data curation and writing–review and editing. **Deanna M. Glassman:** Conceptualization, data curation, writing–review and editing, and funding acquisition. **Amir A. Jazaeri:** Resources, data curation, and writing–review and editing. **Jose A. Rauh-Hain:** Resources, data curation, and writing–review and editing. **Sanghoon Lee:** Conceptualization, methodology, resources, data curation, writing–review and editing, supervision, and project administration. **Bryan M. Fellman:** Methodology, formal analysis, writing–original draft, and writing–review and editing. **Zhenlin Ju:** Conceptualization, methodology, formal analysis, writing–original draft, writing–review and editing, and visualization. **Yuexin Liu:** Conceptualization, methodology, formal analysis, writing–review and editing, and visualization. **Nicole D. Fleming:** Conceptualization, methodology, resources, data curation, and writing–review and editing. **Anil K. Sood:** Conceptualization, methodology, investigation, resources, data curation, writing–original draft, writing–review and editing, visualization, supervision, project administration, and funding acquisition.

ACKNOWLEDGMENTS

We thank The University of Texas MD Anderson Precision Oncology Decision Support and The University of Texas MD Anderson Molecular and Clinical Data Integration Platform teams for their research support. We thank Amy Ninetto, Scientific Editor, Research Medical Library, The University of Texas MD Anderson Cancer Center, for editing the manuscript. This research was supported in part by the National Institutes of Health (CA217685, CA209904, CA101642, and CA016672), The University of Texas MD Anderson Ovarian Cancer Moon Shot, The University of Texas MD Anderson Ovarian Cancer SPORE, the American Cancer Society, the Ovarian Cancer Research Alliance, the Dunwoody Fund, the Khalifa Foundation, the Gordon Fund, and the Frank McGraw Memorial Chair in Cancer Research.

CONFLICT OF INTEREST STATEMENT

Nicole D. Fleming reports consulting fees from GlaxoSmithKline and Immunogen. Amir A. Jazaeri reports consulting fees from Adicet Bio, Alkermes, Inc, Gerson Lehrman Group, Guidepoint, Macrogenics, NuProbe, and Theolytics and stock and/or stock options with Avenge Bio and Greenfire Bio. Jose A. Rauh-Hain reports fees from the National Cancer Institute and Shanghai Xiye Health Consulting Co. Anil K. Sood reports consulting fees from AstraZeneca, Biopath, GlaxoSmithKline, Iylon, Kiyatec, Merck, and Onxeo, grant funding from the National Cancer Institute, and holds a patent for EGFL6 antibody. Shannon N. Westin reports consulting fees from Agenus, AstraZeneca, Caris, Clovis Oncology, Inc, Eisai, Eli Lilly and Company, EQRX, Genentech, GlaxoSmithKline, Immunogen, Merck, Mereo, Mersana, NGM Bio, Nuvectis, Roche, Seagen Inc, Vincerx, and Zentalis and grant funding from AstraZeneca, AvengeBio, Baye, Bio-Path, Clovis Oncology, Inc, Genentech, GlaxoSmithKline, Mereo, Novartis, OncXerna and Zentalis. Timothy A. Yap reports consulting fees from AbbVie, Acrivon, Adagene, Aduro, Almac, Amphista, Artios, AstraZeneca, Athena, Atrin, Avoro, Axiom, Baptist Health Systems, Bayer, BeiGene, Ltd, Boxer,Bristol-Myers Squibb, C4 Therapeutics, Calithera, Cancer Research UK, Clovis Oncology, Inc, Cybrexa, Dizusion, EMD Serono, F-Star, Genmab, Glenmark, GLG, Globe Life Sciences, GSK, Guidepoint, I-Mab, Idience, Ignyta, ImmuneSensor, Institut Gustave Roussy, Intellisphere, Jansen, Kyn, MEI Pharma, Merck, Mereo, Natera, Nexys, Novocure Inc, OHSU, OncoSec, Ono Pharma, Pegascy, PER, Pifzer, Piper-Sandler, Prolynx, Repare, resTORbio, Roche, Schrodinger, Theragnostics, Varian, Versant, Vibliome, Xinthera, Zai Labs, and ZielBio and grant and/or contract funding from Acrivon, Artios, AstraZeneca, BioNTech, Blueprint, BMS, Constellation, Cyteir, Eli Lilly, Forbius, Genentech, GlaxoSmithKline, Haihe, Ionis, Ipsen, Jounce, Karyopharm, KSQ, Kyowa, Mirati, Novartis, Regeneron, Ribon Therapeutics, Rubius, Sanofi, Scholar Rock, Seattle Genetics, TESARO, Inc, Vivace, and Zenith and stock in Seagen Inc. The other authors declare no conflicts of interest.

ORCID

Deanna M. Glassman https://orcid.org/0000-0003-4591-8272
Jose A. Rauh-Hain https://orcid.org/0000-0001-9985-5954
Yuexin Liu https://orcid.org/0000-0002-0828-4656
Nicole D. Fleming https://orcid.org/0000-0003-1785-4072
Anil K. Sood https://orcid.org/0000-0003-4242-1762

REFERENCES

1. National Cancer Institute. Cancer stat facts: ovarian cancer. Accessed October 14, 2021. https://seer.cancer.gov/statfacts/html/ovary.html
2. Kossai M, Leary A, Scoazec JY, Genestie C. Ovarian cancer: a heterogeneous disease. *Pathobiology*. 2018;85(1-2):41-49. doi:10.1159/000479006
3. National Comprehensive Cancer Network. Ovarian cancer including fallopian tube cancer and primary peritoneal cancer. Accessed October 14, 2021. https://www.nccn.org/guidelines/guidelines-detail?category=1&id=1453

4. Wang Q, Peng H, Qi X, Wu M, Zhao X. Targeted therapies in gynecological cancers: a comprehensive review of clinical evidence. *Signal Transduct Targeted Ther*. 2020;5(1):137. doi:10.1038/s41392-020-0199-6
5. Evans T, Matulonis U. Next-generation sequencing: role in gynecologic cancers. *J Natl Compr Cancer Netw*. 2016;14(9):1165-1173. doi:10.6004/jnccn.2016.0123
6. Jennings LJ, Arcila ME, Corless C, et al. Guidelines for validation of next-generation sequencing-based oncology panels: a joint consensus recommendation of the Association for Molecular Pathology and College of American Pathologists. *J Mol Diagn*. 2017;19(3):341-365. doi:10.1016/j.jmoldx.2017.01.011
7. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inf*. 2009;42(2):377-381. doi:10.1016/j.jbi.2008.08.010
8. Vadas A, Bilodeau TJ, Oza C. The evolution of biomarker use in clinical trials for cancer treatments. 2019. Accessed October 3, 2021. https://www.personalizedmedicinecoalition.org/Userfiles/PMC-Corporate/file/The_Evolution_of_Biomarker_Use_in_Clinical_Trials_for_Cancer_Treatments.pdf
9. Ushijima K. Treatment for recurrent ovarian cancer-at first relapse. *J Oncol*. 2010;2010:497429. doi:10.1155/2010/497429
10. Swisher EM, Kwan TT, Oza AM, et al. Molecular and clinical determinants of response and resistance to rucaparib for recurrent ovarian cancer treatment in ARIEL2 (Parts 1 and 2). *Nat Commun*. 2021;12:2487. doi:10.1038/s41467-021-22582-6
11. Moore KN, Secord AA, Geller MA, et al. Niraparib monotherapy for late-line treatment of ovarian cancer (QUADRA): a multicentre, open-label, single-arm, phase 2 trial. *Lancet Oncol*. 2019;20(5):636-648. doi:10.1016/s1470-2045(19)30029-4
12. Le DT, Kim TW, Van Cutsem E, et al. Phase II open-label study of pembrolizumab in treatment-refractory, microsatellite instability-high/mismatch repair-deficient metastatic colorectal cancer: KEYNOTE-164. *J Clin Oncol*. 2020;38(1):11-19. doi:10.1200/jco.19.02107
13. Berton D, Banerjee SN, Curigliano G, et al. Antitumor activity of dostarlimab in patients with mismatch repair-deficient/microsatellite instability–high tumors: a combined analysis of two cohorts in the GARNET study. *J Clin Oncol*. 2021;39(15_suppl):2564. doi:10.1200/jco.2021.39.15_suppl.2564
14. Drilon A, Laetsch TW, Kummar S, et al. Efficacy of larotrectinib in TRK fusion-positive cancers in adults and children. *N Engl J Med*. 2018;378(8):731-739. doi:10.1056/nejmoa1714448
15. Doebele RC, Drilon A, Paz-Ares L, et al. Entrectinib in patients with advanced or metastatic NTRK fusion-positive solid tumours: integrated analysis of three phase 1-2 trials. *Lancet Oncol*. 2020;21(2):271-282. doi:10.1016/s1470-2045(19)30691-6
16. Jardim DL, Schwaederle M, Wei C, et al. Impact of a biomarker-based strategy on oncology drug development: A meta-analysis of clinical trials leading to FDA approval. *J Natl Cancer Inst*. 2015;107(11):djv253. doi:10.1093/jnci/djv253
17. Janku F, Berry DA, Gong J, Parsons HA, Stewart DJ, Kurzrock R. Outcomes of phase II clinical trials with single-agent therapies in advanced/metastatic non-small cell lung cancer published between 2000 and 2009. *Clin Cancer Res*. 2012;18(22):6356-6363. doi:10.1158/1078-0432.ccr-12-0178
18. Schwaederle M, Zhao M, Lee JJ, et al. Association of biomarker-based treatment strategies with response rates and progression-free survival in refractory malignant neoplasms: a meta-analysis. *JAMA Oncol*. 2016;2(11):1452-1459. doi:10.1001/jamaoncol.2016.2129
19. Nakayama K, Nakayama N, Kurman RJ, et al. Sequence mutations and amplification of PIK3CA and AKT2 genes in purified ovarian serous neoplasms. *Cancer Biol Ther*. 2006;5(7):779-785. doi:10.4161/cbt.5.7.2751
20. Centers for Disease Control and Prevention. United States Cancer Statistics: Data Visualizations. Accessed October 14, 2021. https://gis.cdc.gov/Cancer/USCS/#/Demographics/

SUPPORTING INFORMATION

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

How to cite this article: Foster KI, Shaw KRM, Jin J, et al. Clinical implications of tumor-based next-generation sequencing in high-grade epithelial ovarian cancer. *Cancer*. 2023;129(11):1672-1680. doi:10.1002/cncr.34724