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Research Article

Limited Independent Follow-Up with Germline Testing of Variants Detected in *BRCA1* and *BRCA2* by Tumor-Only Sequencing

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ABSTRACT

Introduction: Genomic profiling is performed in patients with advanced or metastatic cancer, in order to direct cancer treatment, often sequencing tumor-only, without a matched germline comparator. However, because many of the genes analyzed on tumor profiling overlap with those known to be associated with hereditary cancer predisposition syndromes (HCPS), tumor-only profiling can unknowingly uncover germline pathogenic (P) and likely pathogenic variants (LPV). In this study, we evaluated the number of patients with P/LPVs identified in *BRCA1* and *BRCA2* (*BRCA1/2*) via tumor-only profiling, then determined the germline testing outcomes for those patients. **Methods:** A retrospective chart review was performed to identify patients with *BRCA1/2* variants on tumor-only genomic profiling, and whether they had germline testing. **Results:** This study found that of 2923 patients with 36 tumor types who underwent tumor-only testing, 554 had a variant in *BRCA1/2* (19.0%); 119 of the 554 patients (21.5%) had a P/LP *BRCA1/2* variant, representing 4.1% of the overall population who underwent genomic profiling. Seventy-three (61.3%) of 119 patients with *BRCA1/2* P/LPV on tumor-only testing did not undergo germline testing, 34 (28.6%) had already had germline testing before tumor-only testing, and 12 (10.1%) underwent germline testing after tumor-only testing. Twenty-eight germline *BRCA1/2* P/LPVs were detected, 24 in those who had prior germline testing, and 4 among the 12 patients who had germline testing after tumor-only testing. **Conclusion:** Tumor-only testing is likely to identify P/LPVs in *BRCA1/2*. Efforts to improve follow-up germline testing is needed to improve identification of germline *BRCA1/2* alterations.

Keywords: BRCA1, BRCA2, germline testing, tumor-only testing

INTRODUCTION

Patients with cancer are often offered genomic testing of their tumors to identify actionable alterations that may be targeted with approved or investigational therapies. There are two commonly used approaches to genomic profiling with next generation sequencing.[1] The first is tumor-normal paired testing, in which tumor tissue and a normal comparator (usually blood, saliva, and sometimes normal tissue) are analyzed concurrently and compared to determine if variants identified originate in the tumor (somatic) or are constitutionally present (germline). The results may be reported, either filtering germline alterations, or by reporting germline alterations separately. The other approach is tumor-only testing, in which it cannot be determined conclusively whether a variant is somatic or germline.[1–3] Many of the genes analyzed within tumors to direct treatment also have significant hereditary cancer implications when pathogenic variants are present in the germline. For example, pathogenic variants (PVs) in *BRCA1* and *BRCA2* identified on tumor-only testing, may be somatic, but they also may be germline, and can be associated with Hereditary Breast and Ovarian Cancer syndrome (HBOC), a genetic condition characterized by an increased lifetime risk of breast, ovarian, pancreatic, and prostate cancer.[4]

Over the past several years, there has been increasing awareness of the likelihood of identifying deleterious germline alterations with tumor-normal paired testing. In studies comparing tumor with normal, varying by panel size and genes under consideration, 3–18% of patients were found to have germline pathogenic/likely pathogenic variants (P/LPVs) in cancer genes, many of which were potentially clinically actionable.[5–13] Of *BRCA1/2*

PVs detected on patient tumor samples, 65–78% were to be of germline origin.[11,14] Based on the high prevalence of germline *BRCA1/2* PVs, National Comprehensive Cancer Network (NCCN) guidelines now recommend that patients with PVs in *BRCA1/2* identified via tumor-only testing receive follow-up germline testing, regardless of tumor type.[15]

In this study, we performed a retrospective chart review to determine the prevalence of P/LPVs in *BRCA1* and *BRCA2* identified by tumor-only testing, the proportion of those patients referred for germline genetic assessment, and the frequency of hereditary *BRCA1/2* PVs detected on germline evaluation. Our results highlight the importance of identifying hereditary alterations in *BRCA1/2* through follow-up germline testing in patients with cancer to guide therapy and predict prognosis.

METHODS

Ethical Approval

This study was performed in accordance with The University of Texas MD Anderson Cancer Center Institutional Review Board (IRB) guidelines, which allowed the IRB to waive the requirement for obtaining written informed consent from the participants.

Patients

We performed a retrospective chart review using a cohort of 2923 patients seen at The University of Texas MD Anderson Cancer Center in Houston, TX, who have received FoundationOne testing, a commercial tumor-only panel. Patients had testing ordered either while a patient at MD Anderson, or before initiating their care at MD Anderson. Patients whose reports were ordered between September 7, 2012, and August 17, 2018, were included.

Patients with *BRCA1/2* variants identified via tumor-only testing that were determined to be P/LPV were subjected to a chart review. Patient demographic data and results of follow-up germline testing were collected. An HBOC-related primary tumor was considered one identified in the breast, ovaries/fallopian tubes, peritoneum, pancreas, or prostate. A positive family history was defined as having a first-degree or two second-degree relatives with any HBOC-related cancer, any female relative within three degrees of relation with ovarian cancer, or any male relative within three degrees of relation with breast cancer. The first NCCN recommendation for germline testing in patients with "*BRCA1/2* mutation detected by tumor profiling in the absence of germline mutation analysis" was published on December 7, 2016, therefore this date was used as cutoff for the NCCN guideline change.[15]

Variant Interpretation

Variant interpretation was performed according to an algorithm depicted in Figure 1. The first source used to interpret variants was ClinVar, a database for germline variant interpretation, publicly available through the National Institutes of Health (NIH) (https://www.ncbi.nlm.nih.gov/clinvar/). Interpretations were recorded when available. Any variants that were reported by ClinVar as "conflicting interpretation" between P/LPV and VUS (variant of uncertain significance) or benign were recorded as such. If there were no interpretation data available through ClinVar, a general algorithm was applied to classify the remaining variants. Truncating mutations (e.g., frameshifts, large deletions, and nonsense mutations) not located close to the 3′ end of the protein were determined to be "inferred pathogenic." Missense mutations, intronic variants, or any other variants that were unable to be labeled "inferred pathogenic" mutations were labeled "inferred VUS."

Statistical Analysis

Statistical analysis was performed using Stata software version 13.1 (Stata Corp LP, College Station, TX). Statistical significance was determined using chi-squared test and Mood's median test as appropriate. Statistical significance was assumed at $P < 0.05$. Yates' correction was used when running chi-squared tests for rows with low cell counts (<5).

RESULTS

This study included 2923 patients with FoundationOne tumor molecular testing. Of these, 554 patients (19.0%) were found to have *BRCA1/2* variants noted on their reports. In this subgroup, 432 (78.0%) had variants classified as benign, likely benign, VUS, or inferred VUS (classified as B/LB/VUS/iVUS). Three patients had variants in ClinVar with conflicting interpretations between VUS and pathogenic. Importantly, 119 patients had tumor molecular profiling reports with pathogenic, likely pathogenic, or inferred pathogenic variants (classified as P/LPV) in *BRCA1/2* (Fig. 2). Thus, 21.5% of the *BRCA1/2* variants noted were P/LPVs and, overall 4.1% of the patient population that had undergone tumor-only testing had P/LPVs in for *BRCA1/2*. This cohort of patients with "clinically significant variants" on tumor-only testing as described in standards and guidelines[1] are referred to as patients with P/LPVs throughout the article.

The distribution of primary tumor sites in our large cohort of patients with tumor-only testing ($n = 2923$) is shown in Figure 3A. Next, we compared the group of individuals with P/LPVs identified on tumor-only testing ($n = 119$) with the cohort of 2804 patients without P/LPVs (Table 1 and Supplementary Table 1). P/LPVs in *BRCA1/2* were more prevalent in female patients (5.0%) than male patients (3.0%) ($p = 0.007$). The distribution of primary tumor sites was found to be significantly different between the two cohorts ($p < 0.001$) where cancers that originated in breast ($p < 0.001$) and ovary/fallopian tube/peritoneum ($p < 0.001$) were significantly

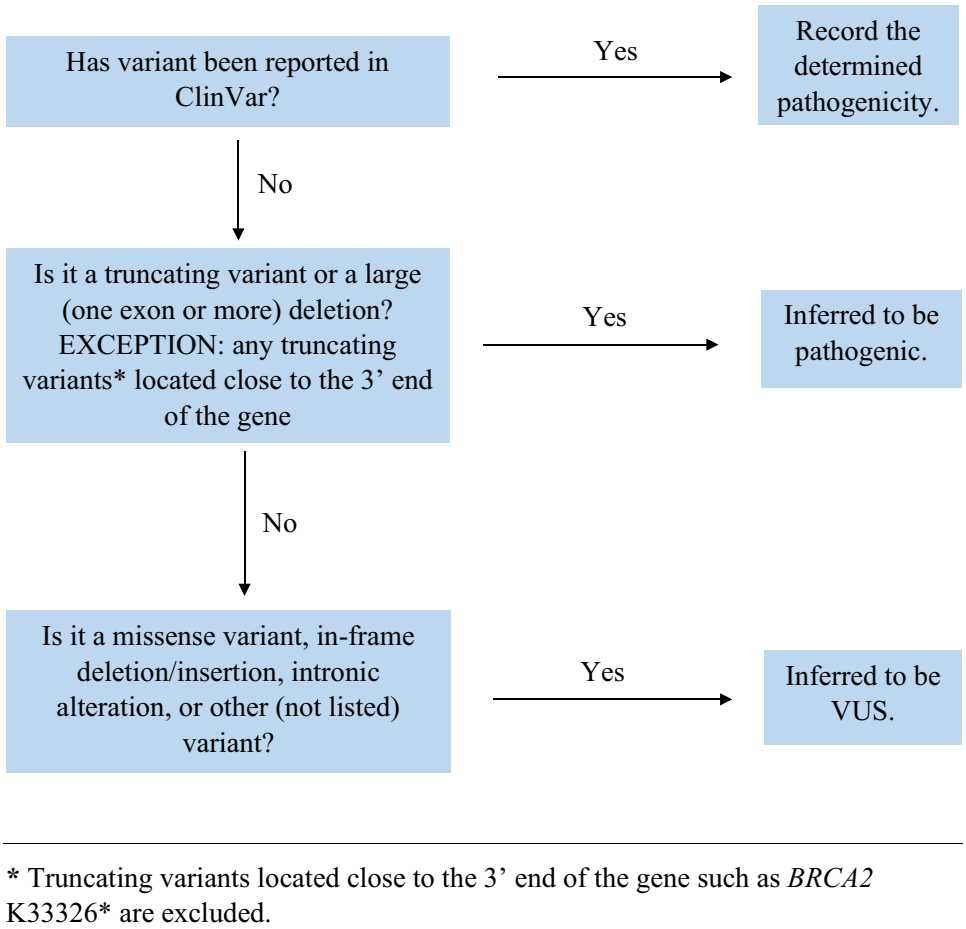

Figure 1. Variant annotation algorithm. VUS, variant of uncertain significance.

overrepresented in the cohort of individuals with P/LPVs when compared with other histologies (Figure 3B). Table 2 and Figure 3B demonstrate comparison of the distribution of primary tumor sites between the cohort of patients with P/LPVs and two other cohorts: patients with B/LB/VUS/iVUS *BRCA1/2* variants (n = 432) and those with conflicting results (n = 3).

Among 119 patients with P/LPVs, 34 (28.6%) had undergone germline testing for *BRCA1/2* before tumor-only testing, and 24 of those were positive (70.6%). Evaluation of the patients without germline testing before tumor-only testing (n = 85) revealed that 12 (14.1%) of them had been referred to germline testing following the assessment of their tumor molecular profiles at our institution. In this cohort of patients who underwent germline testing after tumor-only testing (n = 12), 4 (33.3%) demonstrated a germline P/LPV in *BRCA1/2* (Fig. 2). A review of medical records of these patients with germline P/LPVs (n = 4) indicated that they had been diagnosed with sarcoma of head and neck, cholangiocarcinoma, ovarian cancer, and ampullary cancer.

The cohorts of patients with (n = 12) and without (n = 73) germline testing after tumor-only testing were further characterized in Table 3. A search of medical and public records showed that, in the cohort of 73 patients without germline testing, at least 22 (30.1%) died within the first year following tumor-only testing. At least one of the patients who did not receive germline testing was offered testing by a genetic counselor and declined because of lack of coverage by her insurance company and inability to pay out of pocket for testing. In addition, 15 (20.5%) of the 73 patients were seen only once at our institution, rendering follow-up germline testing at our institution impractical (Table 3).

Changes in the NCCN guideline implemented in December 2016 recommend germline testing for individuals with *BRCA1/2* PVs detected by tumor molecular profiling.[15] In our cohort of 119 patients with P/LPVs in *BRCA1/2*, 99 (83.2%) had tumor-only testing before the guideline change (Table 1). Among the 20 patients tested on or after December 2016, only three (15%) were referred to genetic counseling.

DISCUSSION

Genomic profiling has become a common practice in oncology to guide treatment of patients with advanced solid tumors. Many genes that are analyzed using tumor-only profiling overlap with those associated with HCPS. Given that tumor profiling could be the first or only

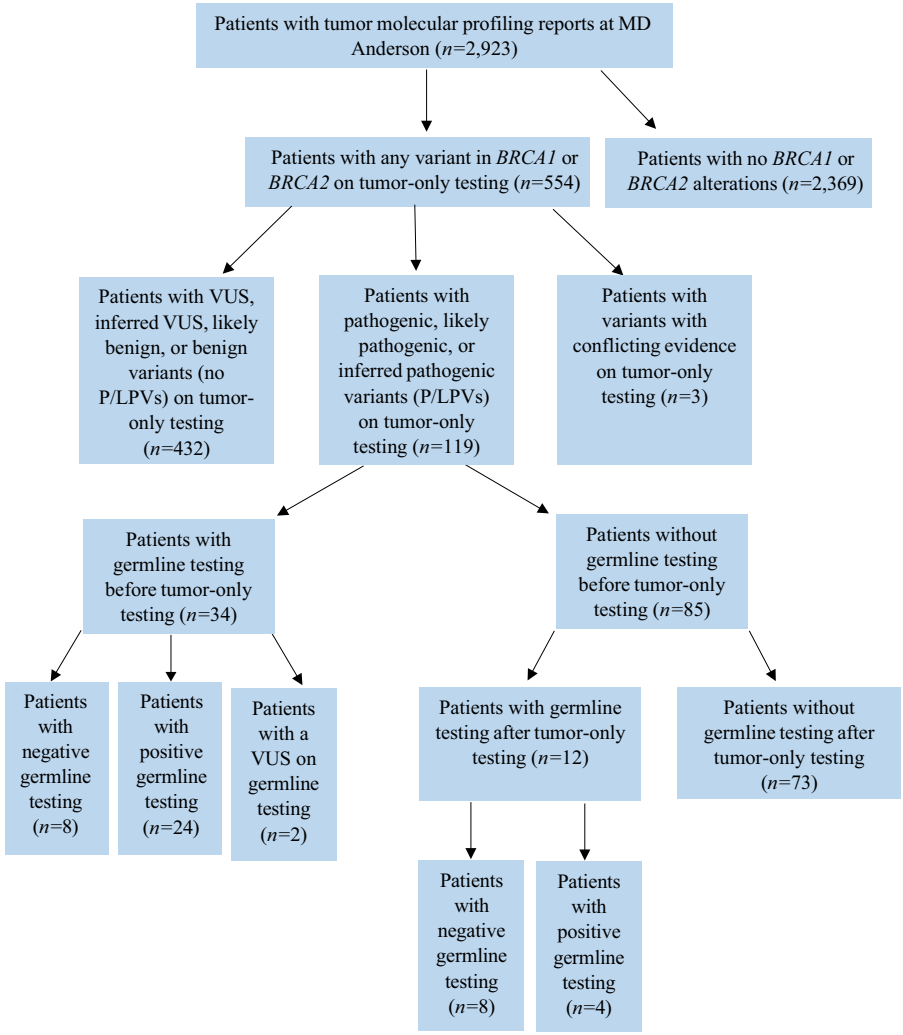

Figure 2. Flowchart of outcomes of tumor molecular profiling. P/LPV, pathogenic/likely pathogenic variant; VUS, variant of uncertain significance.

indication that a patient has an HCPS, it is important to understand the frequency with which that scenario may occur to be able to appropriately counsel patients before genomic testing and manage patients with P/LPVs identified with testing. In this study, we performed a retrospective chart review to characterize cancer patient outcomes of tumor molecular profiling for *BRCA1* and *BRCA2* across 36 cancer types. We identified P/LPVs in *BRCA1/2* in approximately 4% of tumor-only testing reports. Most patients with P/LPVs within our cohort did not undergo germline testing.

Previous studies from our group and others have reported identification of clinically significant germline alterations in *BRCA1/2* upon tumor-normal matched profiling.[7,11] With growing interest in targeting DNA Damage response alterations, recent studies have highlighted that secondary germline P/LPVs may have therapeutic implications. Cobain et al[16] reported that among 1015 patients who underwent tumor-normal profiling, PGVs were identified in 160 (15.8%), including PVs in *BRCA1/2* that led to genomically matched therapy with poly adenosine diphosphate-ribose polymerase (PARP) inhibitors. Stadler et al[6] reported that of 11,974 patients with variety of tumor types, 17.1% of patients had a germline P/LPV, and 7.1% had a germline P/LPV with therapeutic actionability. Of the patients who received targeted therapy for germline alterations, most had alterations in DNA damage response genes (*BRCA1/2, ATM, PALB2,* or *RAD51C/D*) or mismatch repair. Most patients with germline P/LPV *BRCA1/2* variants who were treated with genomically matched therapy had *BRCA*-associated diseases.

Several studies have indicated that more than 50% of patients with germline PVs in genes associated with HCPS that are identified by these platforms would be missed by current guidelines based on evaluation of personal/family history and pathology.[7,11,17] Lincoln et al[18] reviewed 2023 patients who had germline testing following tumor sequencing, and reported 8.1% of germline PVs were missed by tumor sequencing, thus tumor sequencing should not be considered a substitute for germline sequencing when indicated. Of the patients with PVs in their study, 20% did not meet criteria for germline follow-up testing, suggesting that a more routine germline

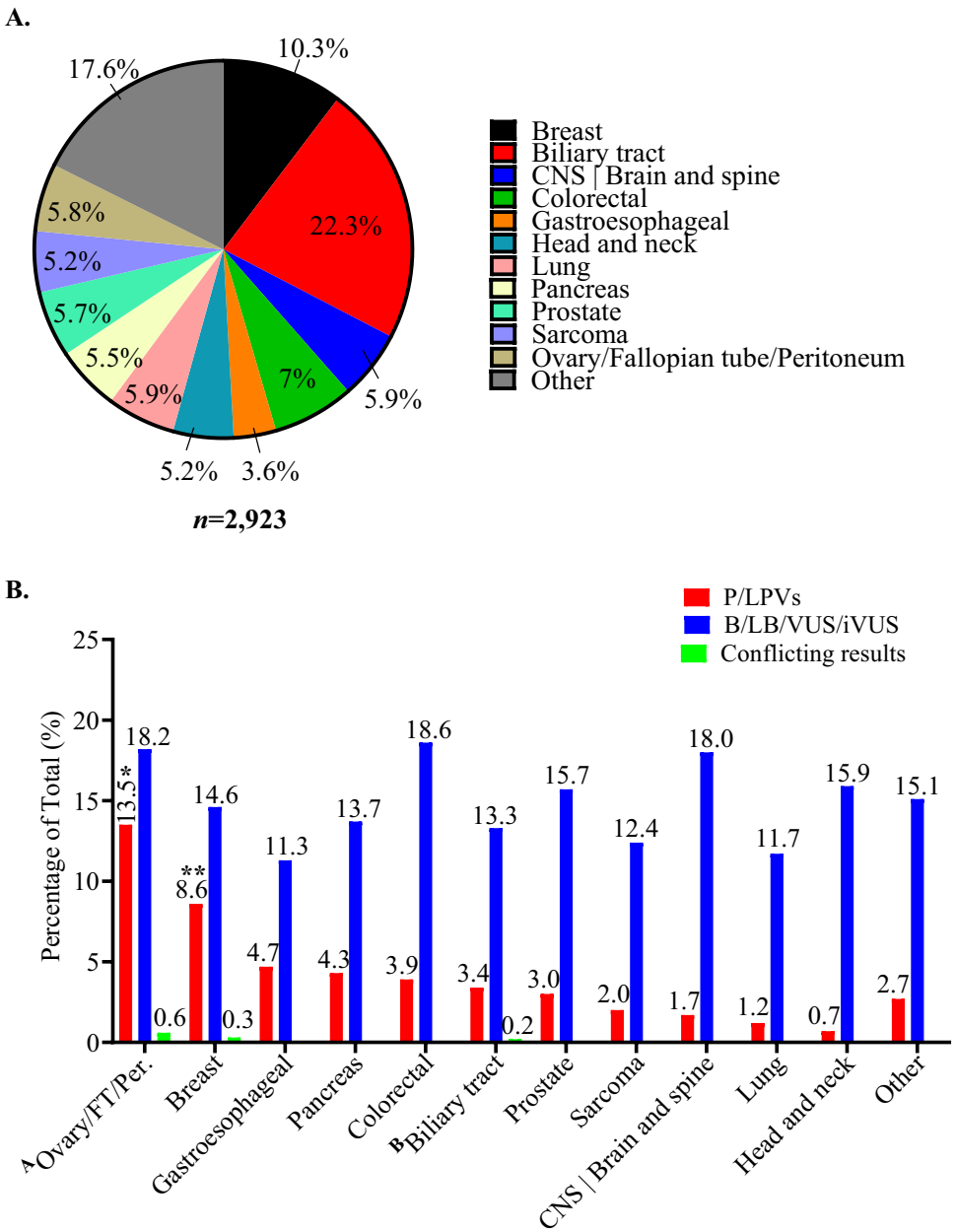

Figure 3. Distribution of primary tumor sites in patients (n = 2923) with tumor-only testing (A) and patients with P/LPVs (pathogenic/likely pathogenic/inferred pathogenic), B/LB/VUS/iVUS (benign/likely-benign/variant of uncertain significance/inferred variant of uncertain significance) variants and conflicting results identified in tumor-only testing (B). Patients with no alterations in *BRCA1/2* were not shown in the bar plot.

[A]Ovarian, fallopian tube and primary peritoneal tumors.

[B]Biliary tract cancers include cholangiocarcinoma, and cancers that originate in gallbladder and ampulla of Vater.

*****Ovarian/Fallopian tube/Peritoneum and ******Breast cancers are overrepresented in the cohort of patients with P/LPVs compared with other histologies.

CNS, central nervous system; FT, fallopian tube.

follow-up plan may be needed to identify most patients with germline P/LPVs. Determination of whether the alteration is somatic or germline may not only have therapeutic implications for the patient but would also have implications for cascade testing and cancer prevention for the patient's family.

NCCN guidelines were amended in December 2016 to recommend germline testing for patients with PVs in *BRCA1/2* identified by tumor sequencing in the absence of germline mutation assessment. The European Society of Medical Oncology Precision Medicine Working Group Germline Subgroup has similarly suggested that patients

Table 1. Characteristics of patients with and without P/LPVs in *BRCA1* or *BRCA2* identified on tumor-only testing

Parameters	Patients With P/LPVs Identified on Tumor-only Testing (*n* =119)	Patients Without P/LPVs* Identified on Tumor-only Testing (*n* = 2804)	*p* Value
Median age at cancer diagnosis (range)	53 (17–79)	55 (2–86)	0.035
Sex, *n* (%)			0.007
Female	79 (5.0)	1510 (95.0)	
Male	40 (3.0)	1294 (97.0)	
Race/Ethnicity, *n* (%)			0.995
White	90 (4.0)	2141 (96.0)	
Black/African American	9 (4.3)	202 (95.7)	
Hispanic	4 (3.1)	125 (96.9)	
Asian	6 (4.4)	129 (95.6)	
Hawaiian/Pacific Islander	1 (6.3)	15 (93.7)	
Native American	0 (0)	3 (100.0)	
Other	4 (5.2)	73 (94.8)	
Unknown	5 (4.1)	116 (95.9)	
Primary tumor site, *n* (%)			<0.001
Breast	26 (8.6)	276 (91.4)	<0.001
Biliary tract**	22 (3.4)	630 (96.6)	0.307
CNS | Brain and spine	3 (1.7)	169 (98.3)	0.164
Colorectal	8 (3.9)	196 (96.1)	0.909
Gastroesophageal	5 (4.7)	101 (95.3)	0.732
Head and neck	1 (0.7)	150 (99.3)	0.05
Lung	2 (1.2)	169 (98.8)	0.075
Pancreas	7 (4.3)	154 (95.7)	0.855
Prostate	5 (3.0)	161 (97.0)	0.477
Sarcoma	3 (2.0)	150 (98.0)	0.251
Ovary/Fallopian tube/Peritoneum	23 (13.5)	147 (86.5)	<0.001
Other	14 (2.7)	501 (97.3)	0.087
Timing of tumor molecular profiling with respect to NCCN guideline change, *n* (%)			0.087
Before guideline change	99 (4.4)	2143 (95.6)	
After guideline change	20 (2.9)	661 (97.1)	

Row percentages were used in the construction of this table. *p* values were computed using chi-squared tests to compare patients with P/LPVs in *BRCA1/2* identified on tumor-only testing to the cohort of patients with no P/LPVs. Yates' correction was used when running chi-squared tests for rows with low cell counts (<5).

*Patients without P/LPVs include those with benign, likely-benign, VUS, and inferred VUS variants of *BRCA1/2* identified on tumor-only testing as well as patients with no *BRCA1/2* alterations and those with conflicting results.

**Biliary tract cancers include cholangiocarcinoma, and cancers that originate in gallbladder and ampulla of Vater. Refer to Supplementary Table 2 for more detailed distribution of the tumors.

CNS, central nervous system; NCCN, National Comprehensive Cancer Network; P/LPV, pathogenic/likely pathogenic variant; VUS, variant of uncertain significance.

with *BRCA1/2* PVs on tumor sequencing should undergo germline testing, regardless of patient tumor type and patient age.[2] In this work published by Mandelker et al.[2], the working group tested use of variant allele frequency as a filter (20% for small insertions/deletions, 30% for SNVs), and recommended exclusion from germline-focused tumor analysis of gene/context/age scenarios in which the germline conversion rate is <10%. Thus they recommended germline follow-up on the 27 genes of >10% germline conversion rate. Although their germline conversion rate is high, they did not recommend germline-focused tumor analysis for intermediate penetrance genes, such as *CHEK2* and *ATM*, as strategies are not well agreed regarding management of risk within families. The ESMO working group subsequently further studied the germline conversion rate of filtered tumor-detected variants in 49,264 paired tumor-normal samples.[19] In this work published by Kuzbari et al,[19] for genes such as *BRCA1, BRCA2,* and *PALB2*, the germline conversion rate in tumor-detected variants was >80%, whereas for genes frequently somatically mutated, such as *TP53, APC,* and *STK11,* the germline conversion rate was < 2%. The ESMO working group updated their recommendations around germline follow-up of tumor-only sequencing to include (1) revision to 5% for the minimum per-gene germ-conversion rate, (2) inclusion of actionable intermediate penetrance genes *ATM* and *CHEK2*, (3) definition of *BRCA1, BRCA2, PALB2, MLH1, MSH2, MSH6,* and *RET* as the seven 'most actionable' cancer-susceptibility genes in which germline follow-up is recommended regardless of tumor type.

Approximately 80% of the patients in our study had undergone genomic profiling before the NCCN guideline amendment and release of ESMO guidelines.[15]

Table 2. Characteristics of patients by *BRCA1* and *BRCA2* results on tumor-only testing

Parameters	Patients With P/LPVs Identified on Tumor-only Testing (*n* = 119)	Patients With B/LB/VUS/iVUS* Variants Identified on Tumor-only Testing (*n* = 432)	Patients With Conflicting Results** (*n* = 3)	Patients With No *BRCA1/2* Alterations (*n* = 2369)	*p* Value
Median age at cancer diagnosis (range)	53 (17–79)	54 (5–79)	52 (16–55)	56 (2–86)	0.027
Sex, *n* (%)					0.026
Female	79 (5.0)	234 (14.7)	2 (0.1)	1274 (80.2)	
Male	40 (3.0)	198 (14.9)	1 (0.1)	1095 (82.0)	
Race/Ethnicity, *n* (%)					0.377
White	90 (4.0)	327 (14.7)	3 (0.1)	1811 (81.2)	
Black/African American	9 (4.3)	39 (18.4)	0 (0)	163 (77.3)	
Hispanic	4 (3.1)	10 (7.8)	0 (0)	115 (89.1)	
Asian	6 (4.4)	26 (19.3)	0 (0)	103 (76.3)	
Hawaiian/Pacific Islander	1 (6.3)	1 (6.3)	0 (0)	14 (87.5)	
Native American	0 (0)	1 (33.3)	0 (0)	2 (66.7)	
Other	4 (5.2)	7 (9.1)	0 (0)	66 (85.7)	
Unknown	5 (4.1)	21 (17.4)	0 (0)	95 (78.5)	
Primary tumor site, *n* (%)					<0.001
Breast	26 (8.6)	44 (14.6)	1 (0.3)	231 (76.5)	
Biliary tract***	22 (3.4)	87 (13.3)	1 (0.2)	542 (83.1)	
CNS | Brain and spine	3 (1.7)	31 (18.0)	0 (0)	138 (80.2)	
Colorectal	8 (3.9)	38 (18.6)	0 (0)	158 (77.5)	
Gastroesophageal	5 (4.7)	12 (11.3)	0 (0)	89 (84.0)	
Head and neck	1 (0.7)	24 (15.9)	0 (0)	126 (83.4)	
Lung	2 (1.2)	20 (11.7)	0 (0)	149 (87.1)	
Pancreas	7 (4.3)	22 (13.7)	0 (0)	132 (82.0)	
Prostate	5 (3.0)	26 (15.7)	0 (0)	135 (81.3)	
Sarcoma	3 (2.0)	19 (12.4)	0 (0)	131 (85.6)	
Ovary/Fallopian tube/Peritoneum	23 (13.5)	31 (18.2)	1 (0.6)	115 (67.6)	
Other	14 (2.7)	78 (15.1)	0 (0)	423 (82.1)	

Row percentages were used in the construction of this table. *p* values were computed using chi-squared tests.
*B/LB/VUS/iVUS denotes benign, likely-benign, VUS (variant of uncertain significance) and inferred VUS variants of *BRCA1* and *BRCA2* identified on tumor only testing.
**"Patients with conflicting results" cohort was disregarded in the statistical analysis because of extremely small sample size (*n*=3).
***Biliary tract cancers include cholangiocarcinoma, and cancers that originate in gallbladder and ampulla of Vater.
CNS, central nervous system; P/LPV, pathogenic/likely pathogenic variant.

However the low genetic testing rates that persisted after NCCN guideline change call into question whether or not this change in NCCN guidelines is common knowledge to all providers regularly ordering tumor sequencing, as this recommendation is listed only in the Genetic/Familial High-Risk Assessment: Breast and Ovarian guidelines. It is possible that providers who do not specialize in breast and/or ovarian cancer may be unaware of this recommendation. Previous studies have identified the need for proper clinical infrastructure when providers are ordering genetic testing.[20,21] Therefore, establishment of a counseling relationship between providers ordering tumor sequencing and clinical genetics experts is necessary. Providers may also consider regularly evaluating patients with tumor sequencing to determine if they are appropriate candidates for germline testing. Another approach may be implementation of an automatic referral pipeline for genetic testing. Clark et al[22] reported that 81 (3.5%) of 2308 patients undergoing tumor sequencing were identified by an automatic referral pipeline; 31 of those patients underwent germline testing and 23 (72% of those tested upon automatic referral) had confirmed PVs.

There are a number of limitations to our study. First, because of the nature of a retrospective chart review, there is inherently a limitation due to missing or incomplete data in the patient's chart. For example, it is possible that patients have received germline testing at outside institutions that are not recorded in their charts; we do not know how many patients were affected by this limitation. Second, this study was performed at a single institution using a cohort that consisted mostly of patients with metastatic or advanced cancer, therefore the results may not be widely generalizable for all patients who receive genomic profiling. Third, even among patients with advanced cancer, there may have been selection bias for genomic testing, and differences even for choice of platform for testing. Fourth, we focused this study on tumor-only testing; however, some of these considerations also apply to identification of mutations in hereditary cancer genes on liquid biopsies with circulating free DNA testing. Last, because our cohort mostly includes patients who have received tumor-only testing before the NCCN guideline changes, it may not properly represent the new practices of physicians and ordering providers.

Table 3. Characteristics of patients with *BRCA1* or *BRCA2* P/LPVs who underwent or did not undergo germline testing following tumor-only testing

Parameters	Patients With Germline Testing After Tumor-only Testing (*n* = 12)	Patients Without Germline Testing After Tumor Only Testing (*n* = 73)	*p* Value
Median age at cancer diagnosis (range)	51 (41–75)	56 (17–79)	0.265
Sex, *n* (%)			0.563
Female	6 (12.2)	43 (87.8)	
Male	6 (16.7)	30 (83.3)	
Race/Ethnicity, *n* (%)			0.970
White	10 (15.4)	55 (84.6)	
Black/African American	0 (0)	6 (100.0)	
Hispanic	0 (0)	1 (100.0)	
Asian	1 (20.0)	4 (80.0)	
Other	1 (25.0)	3 (75.0)	
Unknown	0 (0)	4 (100.0)	
Primary tumor site, *n* (%)			0.632
Associated with HBOC	3 (10.0)	27 (90.0)	
Not associated with HBOC	9 (16.4)	46 (83.6)	
Timing of tumor molecular profiling with respect to NCCN guideline change, *n* (%)			0.847
Before guideline change	9 (13.0)	60 (87.0)	
After guideline change	3 (18.8)	13 (81.2)	
Patient status at the institution, *n* (%)			0.938
One-time consult only	2 (11.8)	15 (88.2)	
Returned for oncology follow-up	10 (14.7)	58 (85.3)	
Family history, *n* (%)			0.623
Significant	7 (15.9)	37 (84.1)	
Not significant	5 (12.2)	36 (87.8)	
Tumor-only report annotation of clinically significant variant, n (%)*			0.617
Actionable	9 (16.7)	45 (83.3)	
VUS	2 (20.0)	8 (80.0)	
Indeterminate	1 (4.8)	20 (85.2)	

Row percentages were used in the construction of this table. *p* values were computed using chi-squared tests to compare cohorts of patients with germline testing after tumor-only testing and patients without germline testing. Yates' correction was used when running chi-squared tests for rows with low cell counts (<5).

*More than one clinically significant variant was reported for one patient.

**Not all patients saw a genetic counselor at the University of Texas MD Anderson Cancer Center.

HBOC, Hereditary Breast and Ovarian Cancer syndrome; NCCN, National Comprehensive Cancer Network; P/LPV, pathogenic/likely pathogenic variant; VUS, variant of uncertain significance.

However, the frequency of patients who received tumor sequencing and have PVs in *BRCA1/2*, as well as the proportion of patients with germline PVs, are in agreement with previous studies.[7,11,23]

These results do indicate, however, that a significant proportion of patients has not received germline testing and/or has not been referred for genetic counseling despite having P/LPV *BRCA1/2* mutations on tumor sequencing. There could be many factors that affect this outcome, some of which are related to the patient's personal situation and cannot be controlled. More than 60% of patients within our cohort died in the first 2 years following tumor-only sequencing, indicating that there may not have been sufficient time for the patients to receive genetic counseling and germline testing.

Many barriers to germline testing were identified during this study. For one, tumor-only testing report *BRCA1/2* interpretations included "in the appropriate clinical context, testing for the presence of germline mutations. . .is recommended"; however, it could be argued that there should be a more prominent indication that germline *BRCA1/2* mutations are common among patients with *BRCA1/2* mutations on genomic reports with stronger statement about the importance of follow-up genetic testing to determine whether the alteration is germline in origin. Furthermore, it is important for laboratories that perform tumor-only sequencing to follow the recommendations put forth by the Human Genome Variation Society (HGVS) when describing variants on reports. HGVS recommends that, in general, "all variants should be described at the most basic level, the DNA level."[24] The use of HGVS nomenclature is not yet standard reporting practice among all laboratories. This can make researching the variant increasingly difficult, as the notation provided can be vague and therefore difficult to search in databases such as ClinVar. Therefore, changes to the lab reports by including HGVS-recommended notation and raising the potential germline nature and need for

germline testing to resolve that could potentially improve follow-up germline testing rates in clinical practice. It would be important to increase provider awareness and create local decision-support tools to ensure appropriate follow-up germline testing is routinely initiated.

In our study, only 21.5% of BRCA1/2 variants identified were a P/LPV *BRCA1/2* variant. This highlights the importance of clearly incorporating expected functional impact of BRCA1/2 variants, when identified. It is also important for ordering physicians to be aware that functional annotations of genetic alterations may evolve and that there are different expectations for updating tumor-only sequencing reports and germline testing reports. A consensus position paper by the Association of Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists recommends that tumor sequencing results "should be static, and the date of issue should be clearly presented."[1] Therefore, it should not be expected of laboratories to update results of tumor sequencing, and providers are expected to remain educated about the changes in medical knowledge. The importance of this duty by providers can be illustrated when considering the implications of the NM_000059.3:c.9976A>T (p.K3326Ter) variant in *BRCA2*. Although this variant is truncating, it is a relatively common variant currently classified as benign. Older tumor-only testing reports evaluated in this study were observed to list the *BRCA2* K3326Ter variant as pathogenic. Continuing education regarding tumor-only testing results is recommended for providers ordering this testing.

Last, most external laboratory results are transmitted to ordering physicians as a PDF or as a hard copy. This in itself is a potential barrier to care, as PDF files (and clearly hard copies) cannot be searched through text recognition. Ideally, such results could be uploaded directly to an electronic health record in a manner that is immediately searchable and interacts with the rest of the database. This way, if a pathogenic variant is identified, there could be an alert, action, or task sent to the ordering physician indicating that a referral should be placed for genetic counseling or germline testing should be pursued for this patient. A fully functional electronic health record could benefit the providers and therefore the patients by making it easier to recognize the next steps in patient care.

CONCLUSION

In summary, our study identified P/LP *BRCA1/2* variants in approximately 4% of tumor-only sequencing reports retrospectively. As expected, the prevalence of *BRCA1/2* P/LPVs differed by tumor type and age at cancer diagnosis. Most patients with *BRCA1/2* P/LPVs identified on tumor sequencing were not referred for germline testing. Further work is needed to enhance education and establish workflows to ensure all patients with P/LP *BRCA1/2* variants identified on tumor-only sequencing receive appropriate follow-up care.

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The work presented was partly based on the master's thesis by Carol Nowlen.[25]

Data Availability

De-identified data will be provided to qualified researchers who engage in independent, original, and rigorous research upon request. Eligible researchers who contact the corresponding author for the de-identified dataset will be required to obtain ethical approval and sign a Data Sharing Agreement (DSA).

Supplemental Material

Supplemental materials are available online with the article.

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