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Identifying Pathogenic Variants in Hereditary Cancer Syndrome Genes via Tumor Molecular Profiling

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IDENTIFYING PATHOGENIC VARIANTS IN HEREDITARY CANCER SYNDROME GENES VIA TUMOR MOLECULAR PROFILING

A

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Tumor molecular profiling is often performed in order to direct cancer treatment options. However, because many of the genes analyzed on tumor molecular profiling overlap with genes known to be associated in the germline with hereditary cancer predisposition syndromes, tumor molecular profiling can unknowingly uncover germline predisposition to cancer development. In this study, we determined the number of patients with pathogenic variants (PVs) identified in *BRCA1* and *BRCA2* (*BRCA1/2*) via tumor molecular profiling at The University of Texas MD Anderson Cancer Center, then performed a retrospective chart review to determine the proportion of such patients that received germline testing and had germline PVs identified. We found that 3.78% (13/2,990; 95% CI 3.09-4.46%) of tumor-only testing reports identified PVs in *BRCA1/2*, 38.94% (44/113; 95% CI 29.95-47.93%) of patients with pathogenic variants in *BRCA1/2* had germline testing, and 63.64% (28/44; 95% CI 49.42-77.85%) of patients with germline testing had germline PVs in *BRCA1/2*. Patients with cancer diagnoses related to *BRCA1/2* were more likely to have had germline testing (72.73% of patients with testing had HBOC-related tumors vs. 36.23% of those without testing, $p < 0.001$). Efforts to improve testing yield should focus on increasing awareness and availability of germline testing for advanced cancer patients with tumor-identified *BRCA1/2* mutations, particularly in non-*BRCA1/2* associated cancer types.

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INTRODUCTION

Tumors are often subject to genetic testing in order to obtain a somatic mutation profile that can influence treatment. There are two broad categories of tumor molecular profiling methods. The first method is tumor-normal paired testing, in which tumor tissue and unaffected tissue are analyzed concurrently and compared to determine if variants identified originate in the tumor (referred to as somatic for the remainder of the paper) or are constitutionally present (hereafter referred to as "germline"). The other is tumor-only testing, in which it cannot be determined conclusively whether a variant is somatic or germline. 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, *BRCA1* and *BRCA2* are commonly analyzed in tumor samples, because targeted therapies such as PARP inhibitors may be considered for tumors with variants identified in *BRCA1/2*. However, pathogenic variants in *BRCA1* and *BRCA2* also cause Hereditary Breast and Ovarian Cancer syndrome (HBOC) when they are present in the germline. HBOC is a genetic condition characterized by an increased lifetime risk of breast, ovarian, pancreatic, and prostate cancer. Given the overlap between genes that are analyzed in tumor profiling and genes that are associated with hereditary cancer predisposition syndromes (HCPS), tumor profiling can unknowingly report tumor-detected mutations that in fact represent an underlying HCPS.

Studies of tumor-normal paired testing have provided insight into how often tumor-detected mutations are present in the germline. In a group of unselected cancer patients who underwent tumor-normal paired testing, approximately 3% of patients had germline pathogenic variants (PVs) associated with HCPS[3]. In a study performed at MD Anderson Cancer Center, 4.3% of patients were found to have germline PVs in 19 genes associated with high penetrance HCPS, following concurrent analysis of tumor and normal tissue in a cohort of advanced cancer patients[7]. A study performed with a similar cohort at Memorial Sloan Kettering revealed that 17.5% of patients with advanced cancer had clinically actionable

germline PVs in 76 genes of low, moderate, or high cancer risk that were identified using tumor-normal paired testing[5].

Studies have also determined how often germline PVs in specific genes are identified following tumor profiling. It has been established through tumor-normal paired testing that mutations in the genes *TP53, APC,* and *PTEN* are commonly somatically mutated in an unselected cohort of advanced cancer patients, as 86.6% of variants with clinical significance identified across 1,000 tumors were identified in one of these three genes, and only 2.8% were germline[7]. This same study also established that 77.78% of pathogenic variants in *BRCA1/2* detected on tumor molecular profiling were germline in origin[7]. Another study published in the same year supported this evidence by demonstrating that 64.5% of patients with pathogenic variants identified in *BRCA1/2* in breast cancer tissue were germline[9]. This led to a change in NCCN guidelines recommending that PVs in *BRCA1/2* identified via tumor-only testing receive follow-up germline testing[12].

Tumor molecular profiling may be the first indication that a patient has HCPS, as many current guidelines that are based on evaluation of personal history, family history, and pathology are missing a significant number of patients with HCPS. Several studies have indicated that over 50% of patients identified by tumor-normal paired testing with germline PVs in genes associated with HCPS would be missed by current guidelines based on evaluation of personal and family history[5,7]. Additionally, a population based germline sequencing study of *BRCA1* and *BRCA2* that was performed by Geisinger revealed that approximately 50% of all patients with identified pathogenic or likely pathogenic variants did not meet current clinical guidelines based on personal and family history[6].

While tumor-normal paired testing readily distinguishes somatic from germline PVs, tumor-only testing is more commonly used in the clinical setting in order to limit cost and turnaround time[8]. Because the germline is contained in the tumor DNA, it is important to understand what type of information patients may receive from tumor-only testing to ensure that they are being adequately informed and

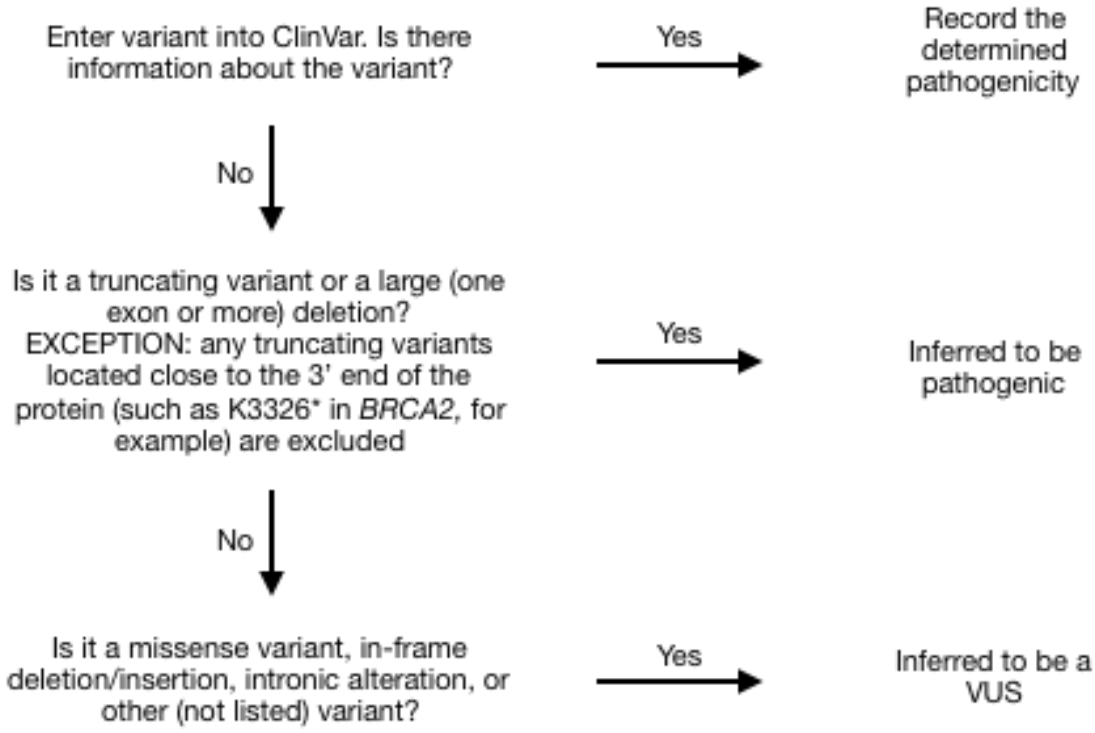
consented[4,8]. There are few studies to date analyzing the identification of HCPS following tumor-only testing. In one such study, Catenacci et al. evaluated how frequently patients that underwent tumor profiling via FoundationOne tumor profiling panel were found to have germline PVs[2]. Seven of 111 patients that were deemed "high risk" after their tumor profiling subsequently received follow-up germline testing. Three had germline PVs, all of which were identified in *BRCA2*. Therefore, 2.7% of the initial cohort that underwent tumor-only testing was found to have a pathogenic germline variant. Because this study was limited in its size and scope, more research on the subject is warranted.

Because studies using tumor-normal paired testing have shown that patients with germline PVs are being missed by clinical guidelines for germline testing, and because tumor-only testing is a more commonly utilized tumor analysis platform, it is paramount to patient care that the hereditary cancer implications of tumor-only testing are well understood. We performed a retrospective chart review to determine how frequently pathogenic variants are identified in *BRCA1* and *BRCA2* via tumor-only testing, what proportion of those patients receive germline testing, and how often the germline testing identifies pathogenic variants in *BRCA1/2*.

METHODS

We performed a retrospective chart review for a cohort of 2,991 patients seen at The University of Texas MD Anderson Cancer Center in Houston, TX that have received FoundationOne testing. Patients with reports available that were ordered from September 7, 2012, to August 17, 2018 were included in the study. The first NCCN recommendation for germline testing for *BRCA1/2* 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 the cutoff for the NCCN guideline change[12].

Figure 1. Variant calling algorithm



Variant interpretation was established according to an algorithm established by the group (Figure1). The first source used to interpret variants was ClinVar, a database for germline variant interpretation, publicly available through the NIH (https://www.ncbi.nlm.nih.gov/clinvar/). Interpretations were recorded when available. Any variants that were reported by ClinVar as "Conflicting Interpretation" between pathogenic/likely pathogenic and VUS or benign were recorded as such. If there was 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."

Patients with variants identified via tumor-only testing that were determined to be pathogenic were subjected to a chart review. Information was collected about demographic information and follow-up germline testing. An HBOC-related primary tumor was considered one identified in the breast,

ovaries/fallopian tubes, peritoneum, pancreas, or prostate. A significant family history was defined as a first-degree relative 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. Statistics were performed using Stata[11]. Statistical significance was determined using chi squared analysis or t-tests. Significance was assumed at $p < 0.05$, and statistics were calculated at a 95% confidence interval.

RESULTS

This study included 2,990 patients with tumor-only testing. Of these, a subgroup was identified in which 457 patients (457/2,990; 15.28%; 95% CI 13.99-16.57%) were found to have any *BRCA1/2* variants noted on the report. In this subgroup, 341 (341/457; 74.62%; 95% CI 70.63-78.61%) had variants classified as benign, likely benign, VUS, or inferred VUS. Three patients had variants interpreted in ClinVar as conflicting between VUS and pathogenic (3/457; 0.66%; 95% CI 0.136-1.91%). A total of 113 patients had tumor molecular profiling reports with pathogenic, likely pathogenic, or inferred pathogenic variants in *BRCA1/2* (113/2,990; 3.78%; 95% CI 3.09-4.46%). This group will be referred for the remainder of the paper as patients with clinically significant variants on tumor-only testing, as per proposed standards and guidelines[14]. The group of individuals with clinically significant variants on tumor molecular profiling were compared to the larger cohort of 2,990 patients to identify any areas in which the groups may be statistically significantly different (Table 1). Primary tumor site was found to be significantly different ($p < 0.001$). Of note, 80.53% of the cohort with clinically significant variants on tumor-only testing had testing prior to the NCCN guideline change in December 2016.

In the group of patients with clinically significant variants identified, 44 (44/113; 38.94%; 95% CI 29.95-47.93%) had record of germline testing for *BRCA1/2*, and 28 were positive (28/44; 63.64%; 95% CI 49.42-77.85%). Those that have received germline testing can be further broken down into those that

had germline testing before tumor-only testing, and those that had germline testing after tumor-only testing. Eleven (11/44; 25%; 95% CI 12.21-37.79%) patients received germline testing after their tumor molecular profiling, and 33 (33/44; 75%; 95% CI 62.21-87.79%) received germline testing before (Figure 2).

Figure 2: Flowchart of outcomes of tumor molecular profiling

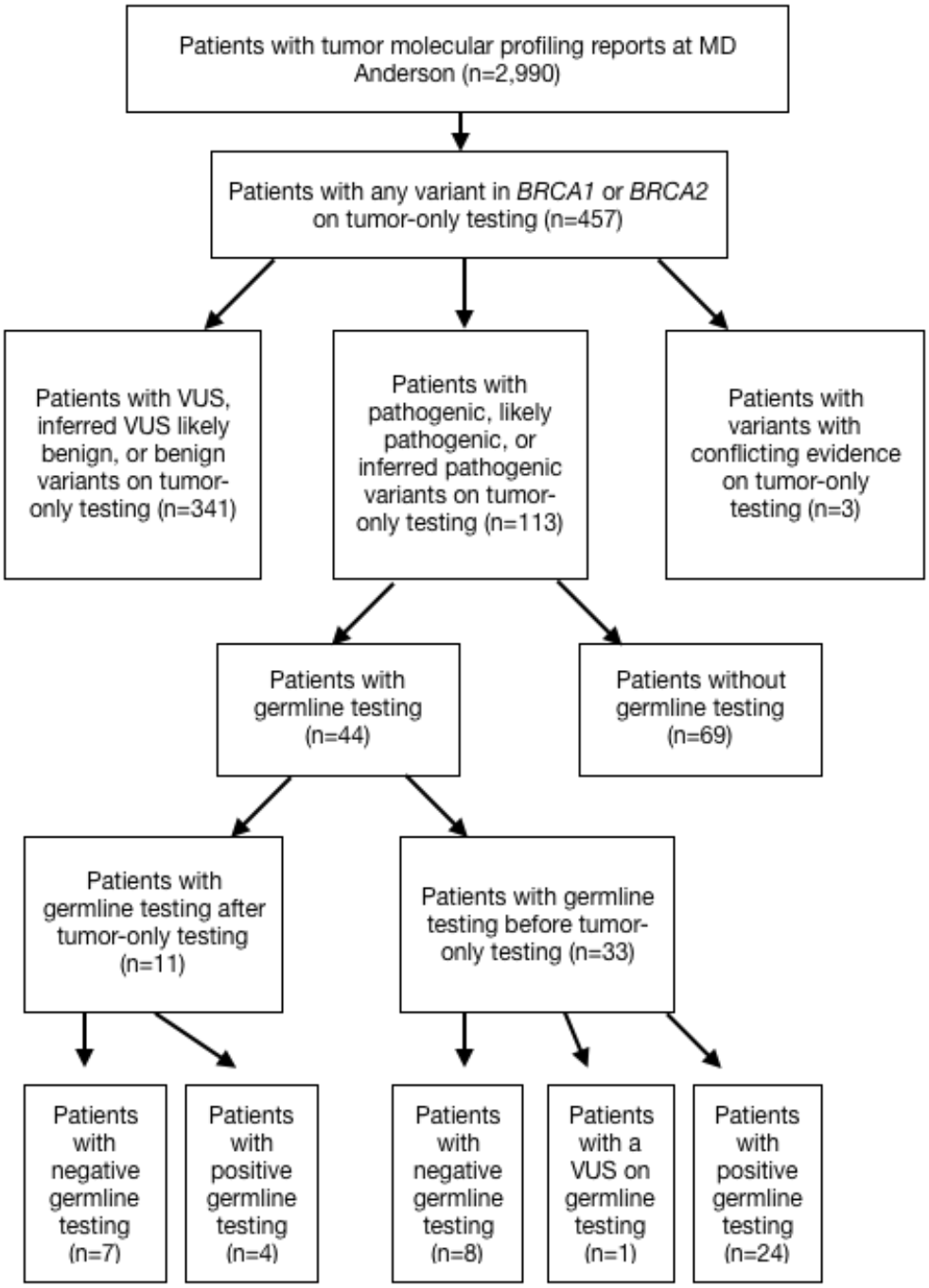


Table 1: Characteristics of patients with pathogenic variants in *BRCA1* and *BRCA2* identified on tumor-only testing

Parameters	Patients with pathogenic variants identified on tumor-only testing (*n* = 113)	Any patient with a tumor-only testing report (n=2,990)	*P*
Median age at cancer diagnosis (range)	53 (17-79)	55 (2-86)	0.6535
Sex, *n* (%)			0.051
Female	74 (65.49)	1,624 (54.33)	
Male	39 (34.51)	1,362 (45.57)	
Unknown	0 (0.00)	3 (0.10)	
Race/Ethnicity, *n* (%)			0.926
White	85 (75.22)	2,284 (76.39)	
Black/African American	10 (7.96)	213 (7.12)	
Hispanic	3 (2.65)	132 (4.41)	
Asian	6 (5.31)	134 (4.48)	
Hawaiian/Pacific Islander	1 (0.88)	16 (0.54)	
Native American	0 (0.00)	3 (0.10)	
Other	4 (3.54)	77 (2.58)	
Unknown	4 (3.54)	131 (4.38)	
Primary tumor site, *n* (%)			< 0.001
Breast	24 (21.24)	294 (9.83)	
Bile duct|Cholangiocarcinoma	15 (13.27)	361 (12.07)	
Ovarian	15 (13.27)	114 (3.81)	
Colorectal	7 (6.19)	117 (5.92)	
Pancreatic	7 (6.19)	112 (3.75)	
Prostate	5 (4.42)	45 (1.51)	
Brain	4 (3.54)	128 (4.28)	
Gallbladder	4 (3.54)	141 (4.72)	
Esophageal	3 (2.65)	56 (1.87)	

Endometrial	3 (2.65)	59 (1.97)
Melanoma	3 (2.65)	46 (1.54)
Sarcoma	3 (2.65)	115 (5.18)
Gastric/Stomach	3 (2.65)	39 (1.30)
Ampullary	2 (1.77)	5 (0.17)
Lung	2 (1.77)	133 (4.45)
Primary peritoneal	2 (1.77)	17 (0.57)
Small intestine	2 (1.77)	17 (0.57)
Renal	2 (1.77)	50 (1.67)
Fallopian tube and adnexal cancer	1 (0.88)	8 (0.27)
Vaginal cancer	1 (0.88)	8 (0.27)
Anal cancer	1 (0.88)	7 (0.23)
Neuroendocrine	1 (0.88)	44 (1.47)
Head and neck	1 (0.88)	135 (4.52)
Bladder	1 (0.88)	28 (0.94)
Skin (non-melanoma)	1 (0.88)	10 (0.33)
Cervical	0 (0.00)	53 (1.77)
Lymphoma	0 (0.00)	17 (0.57)
Thyroid	0 (0.00)	31 (1.04)
Thymus	0 (0.00)	9 (0.30)
Appendiceal	0 (0.00)	4 (0.13)
Leukemia	0 (0.00)	6 (0.20)
Germ cell	0 (0.00)	8 (0.27)
Vulvar	0 (0.00)	4 (0.13)
Carcinoid tumor	0 (0.00)	8 (0.27)
Adrenal cancer	0 (0.00)	7 (0.23)
Cancer of unknown primary	0 (0.00)	35 (1.17)
Other	0 (0.00)	13 (0.43)
Not provided	0 (0.00)	607 (20.30)

Timing of tumor molecular profiling with respect to NCCN guideline change, *n* (%)			0.309
Before guideline change	91 (80.53)	2,227 (74.48)	
After guideline change	22 (19.47)	682 (22.80)	
Unknown	0 (0.00)	81 (2.71)	

P-values from chi-squared tests and t-tests comparing patients with germline testing and patients without germline testing.

The group of people who did not receive germline testing were characterized in more detail and compared to the group that had germline testing (Table 2). Women were significantly more likely to receive testing than men (p=0.012). Patients who were diagnosed at a younger age were also significantly more likely to receive germline testing (p=0.0020), as well as those that had HBOC-related tumors (p>0.001). An informal search of public records determined that, in the group of patients without germline testing, 20 of 69 (28.99%) patients died within the first year following tumor-only testing. Two of the patients that did not receive germline testing were offered it by a genetic counselor and declined. Reasons for declining included difficulty focused around insurance issues, such as difficult communication between laboratories and lack of insurance coverage/inability to pay out of pocket for testing.

Table 2: Comparing patients with germline testing to patients without germline testing

Parameters	Patients with germline testing (n=44)	Patients without germline testing (n=69)	*P*
Age at Diagnosis, median (range)	48 (28-75)	57 (17-79)	0.0020
Sex, *n* (%)			0.012
Female	35 (79.55)	39 (56.52)	
Male	9 (20.45)	30 (43.48)	
Race, *n* (%)			0.290
White	33 (75.00)	52 (75.36)	
Black/ African American	3 (6.82)	7 (10.14)	
Hispanic	3 (6.82)	0 (0.00)	

Asian	2 (4.55)	4 (5.8)	
Hawaiian/Pacific Islander	1 (2.27)	0 (0.00)	
Other	1 (2.27)	3 (4.35)	
Unknown	1 (2.27)	3 (4.35)	
Timing of tumor molecular profiling with regards to guideline change, *n* (%)			0.783
Before guideline change	36 (81.82)	55 (79.71)	
After guideline change	8 (18.18)	14 (20.29)	
Primary tumor site, *n* (%)			<0.001
Associated with HBOC	32 (72.73)	25 (36.23)	
Not associated with HBOC	12 (27.27)	44 (63.77)	
Patient status at the institution, *n* (%)			0.526
One-time consult only	8 (18.18)	16 (23.19)	
Returned for oncology follow-up	36 (81.82)	53 (76.81)	
Family History, *n* (%)			0.258
Significant	12 (27.27)	12 (18.18)	
Not significant	32 (72.73)	54 (81.82)	
Tumor-only report annotation of clinically significant variable, *n* (%)			0.522
Actionable	34 (77.27)	52 (75.36)	
VUS	2 (4.55)	1 (1.45)	
Indeterminate	8 (18.18)	16 (23.19)	
Patient decisions on germline testing when seen by genetic counselor at MD Anderson, *n* (%)			<0.001
Consented	23 (95.85)	0 (0.00)	
Declined	1 (4.17)	2 (100.00)	

P-values from chi-squared tests and t-tests comparing patients with germline testing and patients without germline testing.

DISCUSSION

Tumor molecular profiling is a common practice in oncology to aid in determining treatment plans for patients. Many genes that are analyzed using tumor molecular profiling overlap with genes that are associated with HCPS. Because tumor molecular profiling could potentially be the impetus that prompts a patient to present to genetics, it is important to understand the frequency with which that scenario may occur to be able to provide adequate informed consent. In this study we specifically characterized the patient outcomes of tumor molecular profiling on *BRCA1* and *BRCA2*.

This study found that clinically significant variants in *BRCA1/2* are identified in less than 4% of tumor-only testing reports. Over the course of almost 6 years, only 113 reports with clinically significant variants in *BRCA1/2* were identified which is an average of just under 19 patients per year at a single institution. This figure provides a convincing argument that it is feasible for every patient with a clinically significant variant identified in *BRCA1/2* on tumor-only molecular profiling test to be referred for genetic counseling and consideration of germline testing.

In order to identify some of the reasons that patients may not have received germline testing, the cohort without germline testing was compared to the group with germline testing. First and foremost, almost 80% of the cohort with clinically significant variants identified on tumor-only testing without germline testing received it before the change in NCCN guidelines in December 2016 that recommends that patients with clinically significant variants in *BRCA1/2* identified on tumor-only testing receive follow-up germline testing. Although these patients were occasionally referred for germline testing prior to December 2016 because of their tumor-testing results, they could not always be expected to have been because it was not yet considered standard of care. It should be considered, however, that over 80% of patients with germline testing received tumor-only testing prior to the guideline change, so there are clearly additional factors that are driving germline testing. For example, patients with HBOC-related primary tumors are significantly more likely to receive germline testing than those with tumors not related to HBOC. This calls to question whether or not this change in NCCN guidelines is common

knowledge to all providers regularly ordering tumor molecular profiling tests, as this recommendation is listed only in the Genetic/Familial High-Risk Assessment: Breast and Ovarian guidelines. It is possible that who do not specialize in breast and/or ovarian cancer may be unaware of its existence. Previous studies have identified the need for proper clinical infrastructure when providers are ordering genetic testing[1,10]. It is important that there is an established relationship with providers with clinical genetics expertise for the scenarios in which an ordering physician may not have extensive experience with hereditary cancer predisposition syndromes. Providers may also consider retrospectively evaluating any patients with tumor profiling tests before 2016 to determine if they are appropriate candidates for germline testing.

Other factors could account for the lack of germline testing as well. Over 65% of patients died in the first two years following tumor-only testing, indicating that there may have not been sufficient time for the patients to receive germline testing. Eighteen of the patients that never received testing (18/69, 26.09%) could not have reasonably had germline testing at our institution--16 because they were seen only once at our institution for a one-time visit, and two that saw a genetic counselor and declined germline testing.

There are a number of limitations to our study. Due to the nature of a retrospective chart review, there is inherently a limitation due to missing or incomplete data in the patient's chart. It is possible that patients have received germline testing at outside institutions that are not recorded in their charts, or that they passed away and it had not been reported. Additionally, this study was performed at a single institution in a cohort that consisted mostly of advanced cancer patients, therefore the results may not be widely generalizable for all patients who receive tumor molecular profiling. Lastly, this cohort widely reflects patients that have received tumor-only testing prior to the NCCN guideline changes to include tumor profiling results, so this group may not properly represent the new practices of physicians and ordering providers. However, the number of patients that received tumor molecular profiling and have

germline PVs in *BRCA1/2*, as well as the number of patients with germline PVs, are in agreement with previous studies[2,5,7], indicating that the data is consistent.

These results do indicate, however, that there is a significant number of patients that have not received germline testing despite being candidates. There could be many factors that affect this outcome, some of which are related to the patient's personal situation and cannot be controlled. However, many barriers to germline testing were identified during this study. For one, despite the fact that tumor-only testing reports analyzed include in the interpretation of variants in *BRCA1/2* deemed as "actionable" that "in the appropriate clinical context, testing for the presence of germline mutations...is recommended," it could be argued that there should be a more prominent indication on the test report recommending that the ordering physician refer the patient to genetic counseling if he or she has not already received germline testing. Furthermore, it is important for laboratories who perform tumor-only testing 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. Descriptions at the RNA and/or protein level may be given in addition" and that "all variants should be described in relation to an accepted reference sequence."[13] The use of HGVS nomenclature is not yet standard reporting practice amongst all laboratories. This can make researching the variant increasingly difficult, as the notation provided can be vague and therefore difficult to search in ClinVar. Changes to the reports by indicating more obviously that a referral to genetic counseling is warranted and by including HGVS-recommended notation could potentially boost the proportion of patients that receive follow-up germline testing.

It is also important for ordering physicians to acknowledge the difference between expectations for updating tumor-only testing 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 molecular profiling results "should be static, and the date of issue should be clearly presented."[14] Therefore it should not be expected of laboratories to update results of

tumor molecular profiling, 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*. While this variant is truncating, it is a relatively common variant currently classified as benign. Older tumor-only testing reports in this study were observed to list the *BRCA2* K3326Ter variant as pathogenic.

Lastly, 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 medical record (EMR) 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 EMR could benefit the providers and therefore the patients by making it easier to recognize the next steps in patient care.

In summary, our study identified that less than 4% patients with tumor molecular profiling reports have reported a pathogenic variant in *BRCA1/2*, and approximately one-third of those patients have had germline testing. It is important to note that many of the patients who have not received germline testing received tumor-only testing prior to NCCN guidelines changed to include tumor profiling results, and that over half of the patients without germline testing died within the first two years following tumor profiling. Regardless, due to the fact that almost two thirds of patients with PVs in *BRCA1/2* on tumor molecular profiling have not received testing, there is concern regarding provider education on the NCCN recommendation for follow-up germline testing. Going forward, this problem could be mitigated by more obvious notation on tumor molecular profiling reports indicating that patients with clinically significant variants identified in *BRCA1/2* should receive follow-up, and having providers ordering tumor profiling tests establish clinical infrastructure (such as a relationship with a genetic counselor to

whom they can make referrals) to ensure any patient with a clinically significant variant identified in *BRCA1/2* on tumor molecular profiling receive follow-up germline testing.

APPENDIX

Supplementary Table 1: Germline and somatic variants identified in patients with pathogenic germline variants.

Study ID	Variant(s) identified on tumor-only testing (gene, variant)	Variant identified on germline testing (gene, variant)	Primary tumor site
1	*BRCA2*, A938fs*21; *BRCA2*, A938P	NM_000059.3(BRCA2):c.2808_2811del (p.Ala938Profs)	Breast
2	*BRCA1*, Q1756fs*74; *BRCA2*, P655R	NM_00729.4(BRCA1):c.5266dupC (p.Gln1756Profs)	Breast
4	*BRCA1*, Q804fs*10	*BRCA1, 2529del4*†	Breast
5	*BRCA2*, F1182fs*1	NM_000059.3(BRCA2):c.3545_3546delTT (p.Phe1182Terfs)	Breast
6	*BRCA2*, E49*	NM_000059.3(BRCA2):c.145G>T (p.Glu49Ter)	Breast
7	*BRCA2*, c.7618-1G>A	NM_000059.3(BRCA2):c.7618-1G>A	Breast
9	*BRCA1*, R496H; *BRCA2* S1982fs*22	NM_000059.3(BRCA2):c.391delT (p.Ser131Profs)	Ovarian
10	*BRCA2*, E1646fs*23	NM_000059.3(BRCA2):c.4936_4939delGAAA (p.Glu1646Glnfs)	Breast
11	*BRCA1*, E23fs*17	NM_007294.3(BRCA1):c.68_69delAG (p.Glu23Valfs)	Anal cancer
14	*BRCA2*, Q548*	NM_000059.3(BRCA2):c.1642C>T (p.Gln548Ter)	Breast
16	*BRCA2*, W1692fs*3	NM_000059.3(BRCA2):c.5073dupA (p.Trp1692Metfs)	Breast
18	*BRCA1*, V1736A	NM_007294.3(BRCA1):c.5207T>C (p.Val1736Ala)	Ovarian
19	*BRCA2*, A2717S; *BRCA2*, E881fs*14; *BRCA2*, E880_S884del	*BRCA2, 2869delG*†	Ovarian
20	*BRCA1*, E23fs*17	NM_007294.3(BRCA1):c.68_69delAG (p.Glu23Valfs)	Ovarian
21	*BRCA1*, V340fs*1	NM_007294.3(BRCA1):c.1018delG (p.Val340Terfs)	Ovarian
23	*BRCA1*, E143K; *BRCA2*, N43fs*1; *BRCA2*, V1283fs*2	NM_000059.3(BRCA2):c.3847_3848delGT (p.Val1283Lysfs)	Breast
24	*BRCA2*, H2417fs*3	NM_000059.3(BRCA2):c.7251_7252delCA (p.His2417Glnfs)	Prostate
27	*BRCA1*, S628fs*2	*BRCA1, 1999ins11*†	Ovarian
28	*BRCA1*, M1775R	NM_007294.3(BRCA1):c.5324T>G (p.Met1775Arg)	Breast
29	*BRCA2*, I1151fs*7	*BRCA2, 3678insT*†	Breast
30	*BRCA1*, E143*	NM_007294.3(BRCA1):c.427G>T (p.Glu143Ter)	Breast
34	*BRCA2*, K3326*; *BRCA2*, Y1894FS1	NM_000059.3(BRCA2):c.5681dupA (p.Tyr1894Terfs)	Lung

35	*BRCA2* Y2215fs*10	NM_000059.3(BRCA2):c.6641dupC (p.Tyr2215Leufs)	Pancreas
36	*BRCA1*, Deletion Exon 19; *BRCA1*, Q1327*	NM_007294.3(BRCA1):c.3979C>T (p.Gln1327Ter)	Sarcoma of the head and neck
39	*BRCA2*, L1768fs*5	NM_000059.3(BRCA2):c.5303_5304delTT (p.Leu1768Argfs)	Ampulla
41	*BRCA2*, 2766fs*11	NM_000059.3(BRCA2):c.8297delC (p.Thr2766Asnfs)	Prostate
43	*BRCA1*, D821fs*25, *BRCA1* P1637L	NM_007294.3(BRCA1):c.2457delC (p.Asp821Ilefs)	Pancreas
44	*BRCA1* E23fs*18	NM_007294.3(BRCA1):c.66dupA (p.Glu23Argfs)	Bile Duct| Cholangio-carcinoma

Variants identified on tumor-only testing were recorded as indicated on the test report. All variants identified in *BRCA1/2* on tumor-only testing were included. Germline variants are reported as they currently appear in ClinVar as of April 28, 2019.
†Indicates a germline variant that was unable to be located in ClinVar due to limited information/alternate notation included on the germline testing report. The nomenclature used on the testing report available was maintained.

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