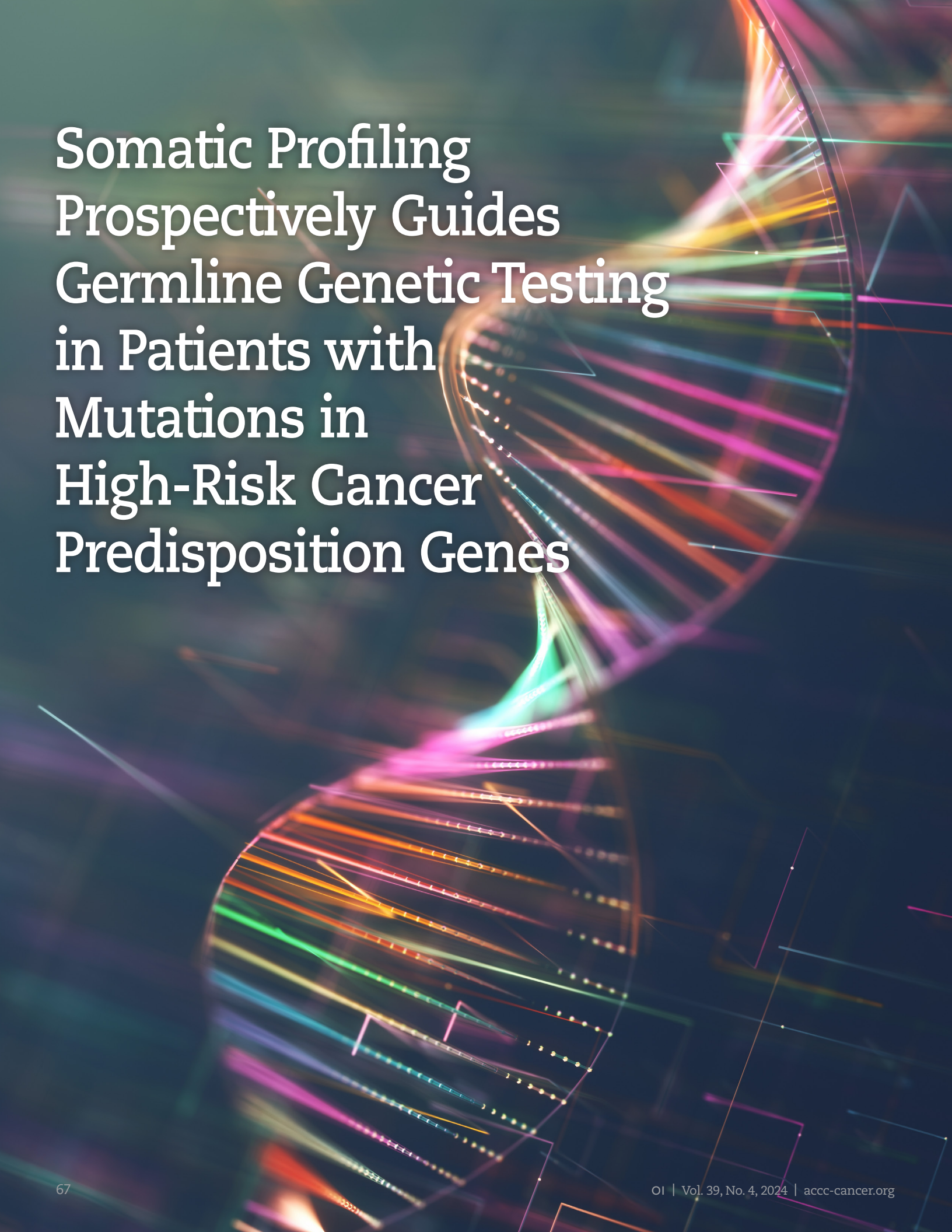

Somatic Profiling Prospectively Guides Germline Genetic Testing in Patients with Mutations in High-Risk Cancer Predisposition Genes

BY SOURAT DARABI, JEANNE P. HOMER, CHELSEY B. TORRES, CARLOS E. ZUAZO, VALENTINA DALILI-SHOAIE, MICHAEL J. DEMEURE, DAVID R. BRAXTON

In Brief

While approximately 15% of patients with advanced cancer have an inherited predisposition to malignancy, many patients who carry a pathogenic germline variant do not qualify for genetic testing based on current clinical guidelines. Offering genetic testing to those patients with the highest likelihood of carrying a germline mutation is preferable to unselected screening. In this study, investigators used a tiered gene list to help identify patients whose somatic mutations were more likely to be found in the germline.

Genes were classified into 3 tiers based on the likelihood of germline pathogenicity. Patients whose routine somatic sequencing tests revealed a mutation that was classified as high-risk (tier 1) were offered participation in this study. Enrolled patients received standard genetic counseling and were offered germline genetic testing via a commercial laboratory next-generation sequencing panel. In all, 9.2% of somatic sequencing tests harbored a high-risk (tier 1) gene mutation; an inherited predisposition to cancer was confirmed in almost 50% of patients enrolled in the study. Patients with gastrointestinal or brain cancers that displayed a high-risk variant were more likely to be confirmed as having a germline mutation than were those with other cancer types. These findings support expanding clinical criteria and lowering barriers for germline genetic testing in patients with advanced cancer.

Approximately 15% of patients with advanced cancer have an inherited predisposition to malignancy, as revealed by germline genetic testing of unselected patients with cancer.[1,2] Despite this genetic predisposition, over one-third of patients who carry a pathogenic germline variant do not qualify for genetic testing based on current clinical testing guidelines.[3–10] Many patients with cancer undergo molecular profiling of their tumor using commercial next-generation sequencing (NGS) laboratories. Most of these laboratories perform tumor-only somatic sequencing without matched germline sequencing analysis, even though variants identified in tumor-only sequencing could be of germline origin.[11] In a study of over 2300 patients whose tumors were profiled, 3.5% were referred for genetic counseling and testing based on the profiling results, and 1.6% were referred based on other concerns.[12] Of these, 74% had confirmed germline pathogenic variants.

Of note, FDA approvals for targeted therapies depend on identification of pathogenic genetic variants that could be only within the tumor or in a patient's germline and often in genes that predispose to inherited forms of cancer.[13–15] In addition to therapeutic implications for the individual, supplementing somatic sequencing with germline testing may reveal familial pathogenic variants, which would extend benefits to family members by informing them about the risk of disease and its prevention or early detection.[16]

For these reasons, supplementing the current practice of somatic sequencing with germline testing of genes associated with hereditary predisposition to cancer would improve the current treatment of cancer and inform on the risk of future cancers.[12] The rapid expansion of NGS into routine clinical practice makes it feasible to integrate these 2 approaches to identify variation in both the cancer and inherited genomes, both of which are clinically important and informative for the treatment and monitoring of patients with cancer.[17] Currently, there are clinical trials that include targeted therapy for patients whose tumors or germline findings show deficiency in the DNA damage repair pathways including mutations in 1 of the mismatch repair genes (*MLH1* [Lynch syndrome], *MSH2*, *MSH6*, or *PMS2*) and clinical indications in homologous recombination deficiency genes (*BRCA1* and *BRCA2* [hereditary breast and ovarian cancer syndrome]).[18–21] In addition, the FDA approved use of talazoparib in combination with enzalutamide for patients with prostate cancer that harbors mutations in *MLH1*.[22]

> Screening all patients with cancer for inherited cancer syndromes is costly, and current genetic testing paradigms suffer from payer constraints and lack of patient throughput.[23]

Screening all patients with cancer for inherited cancer syndromes is costly, and current genetic testing paradigms suffer from payer

constraints and lack of patient throughput.[23] Further, 1 of the most significant barriers to implementing genetic testing is the need to ensure that appropriate patients are selected for testing.[24] Offering genetic testing to patients with the highest likelihood of carrying a germline mutation responsible for a predisposition syndrome is more cost-effective than unselected screening. To develop a selective screening methodology, we conducted a study at the Hoag Family Cancer Institute in Newport Beach, California, with the intent of providing genetic counseling and genetic testing to those patients with cancer whose somatic profiling tests returned mutations associated with hereditary cancer predisposition even if they were not eligible by current clinical or payer guidelines for these clinical services. Here, we describe our experience using a tiered approach for identifying hereditary cancers in patients who have undergone somatic sequencing.

Our Methods

Based on Hoag Family Cancer Institute's criteria for reflex testing and as detailed in our previous studies, patients were identified for tumor testing.[25] Pathologists initiated comprehensive NGS molecular

Table 1. Study Inclusion Criteria and Other Defining Parameters

	Inclusion Criteria	Hoag Family Cancer Institute's Precision Medicine Program Recommendation	Gene List		
High Risk: Tier 1	High to moderate penetrance; onset in children or adults; higher likelihood of germline mutation if found mutated on somatic sequencing panel	**Actionable mutation:** Strongly recommend genetic counseling AND germline confirmation testing	*ATM*[a,b,c]	*BAP1*[a]	*BMPR1A*[d]
			BRCA1[d]	*BRCA2*[d]	*BRIP1*[b]
			MSH2[d]	*MSH6*[d]	*MUTYH*[d]
			DICER1	*PALB2*[b]	*RUNX1*
			SDHAF2[d]	*SDHB*[d]	*SDHC*[d]
			SDHD[d]		
Intermediate Risk: Tier 2	Genes of high to moderate penetrant disorders; genes display high rates of somatic mutation or are typically childhood onset.	**Potentially actionable mutation:** Strongly recommend correlation to family and personal history; if suspicious, genetic counseling and germline confirmation testing if variant is consistent with known or expected pathogenic variants	*APC*[d]	*CDH1*	*MLH1*[d]
			MEN1	*NF1*	*NF2*[d]
			PMS2	*POLE*	*PTEN*[d]
			PTPN11	*RB1*[d]	*RET*[d]
			SMAD4[d]	*SMARCA4*	*STK11*[d]
			TGFBR2[d]	*TSC1*[d]	*TSC2*[d]
			VHL[d]	*WT1*[d]	
Low Risk: Tier 3	Low to moderately penetrant disorders, autosomal recessive (AR) inheritance (carriers unaffected); high rates of somatic-only mutation or onset in neonatal or childhood age.	**Not actionable:** No genetic counseling or confirmatory testing is necessary	*BARD1*	*CHEK2*[c]	*HNF1A*
			FH	*NBN*	*RAD50*
			RECQL4	*TP53*[c,d]	

[a] Truncating type variants are likely the only type of mutation significantly associated with germline mutations.
[b] Germline interpretation is known to be subject to high levels of interlaboratory disagreement.
[c] Founder mutations
[d] Based on American College of Medical Genetics and Genomics secondary findings list, version 2.0.

profiling at the time of diagnosis for selected malignancies. Results were reviewed by study members, and recommendations for genetic counseling were made based on select gene list. We developed a list of cancer predisposition genes by examining the American College of Medical Genetics and Genomics secondary findings list, version 2.0,[26] reconciling for coverage on our somatic NGS assay. We classified the reconciled genes into 3 tiers based on the likelihood of germline pathogenicity when found on somatic profiling panels as determined by prior studies (Table 1).[3–7] The inclusion criteria and other defining parameters are shown in **Table 1**. Patients with variants found by somatic sequencing and suspected to be germline in origin were evaluated using a 3-tier system based on their likelihood of germline origin including founder mutations (ie, genetic alterations with high frequencies within culturally or geographically isolated groups). Patients with tier-1 (high-risk) mutations were recruited to the study by contacting the treating physician.

All somatic sequencing panel results for patients at a single high-volume regional cancer center were prospectively reviewed based on the investigational review board¬–approved protocol (WIRB® Protocol 20191808) for our designated tier-1 genes (*ATM*, *BAP1*, *BMPR1A*, *BRCA1*, *BRCA2*, *BRIP1*, *MSH2*, *MSH6*, *MUTYH*, *PALB2*, *RUNX1*, *SDHAF2*, *SDHB*, and *SDHC*) and known founder mutations in additional genes. Physicians treating patients whose tumors harbored a mutation in 1 of these genes were contacted and, if they agreed, their patients were offered participation in this study. Informed consent was obtained; enrolled patients had genetic counseling and testing for a multigene panel provided by Invitae Corporation at no cost to the patient. Genomic DNA was extracted, and sequence and deletion/duplication analysis of 84 genes performed by the laboratory. Invitae's internal variant classification scheme was used to classify variants.[27] National Comprehensive Cancer Network (NCCN) guidelines for genetic/familial high-risk assessment for cancer detection, prevention, and risk reduction were consulted to identify patients who would not have qualified for testing based on NCCN criteria.

Our Methods

As of May 22, 2023, we reviewed 3979 somatic test results, and 364 (9.1%) patients had tier-1 gene variants or founder mutations in their results. Forty eligible patients (11%) were enrolled, and 39 (97.5%) completed testing with 1 patient declining germline genetic testing. **Figure 1** is an illustration of a patient flow chart that shows the groups of patients in the study based on their eligibility, enrollment, and testing status. All patients who were eligible for enrollment went on to complete testing except for 1 patient (2.5%). Further, 15 eligible patients (37.5%) would not have met the standards for genetic testing based on the latest NCCN criteria at the time of their appointment. Overall, 46% of the enrolled study cohort were male, and 54% were female (average age, 67 years). The most common tier-1 genes identified on somatic testing were *BRIP1*, *BRCA1*, and *BAP1*. Of 364 patients who completed germline testing, 17 patients (4.7%) had

Figure 1. Patient Flow Chart

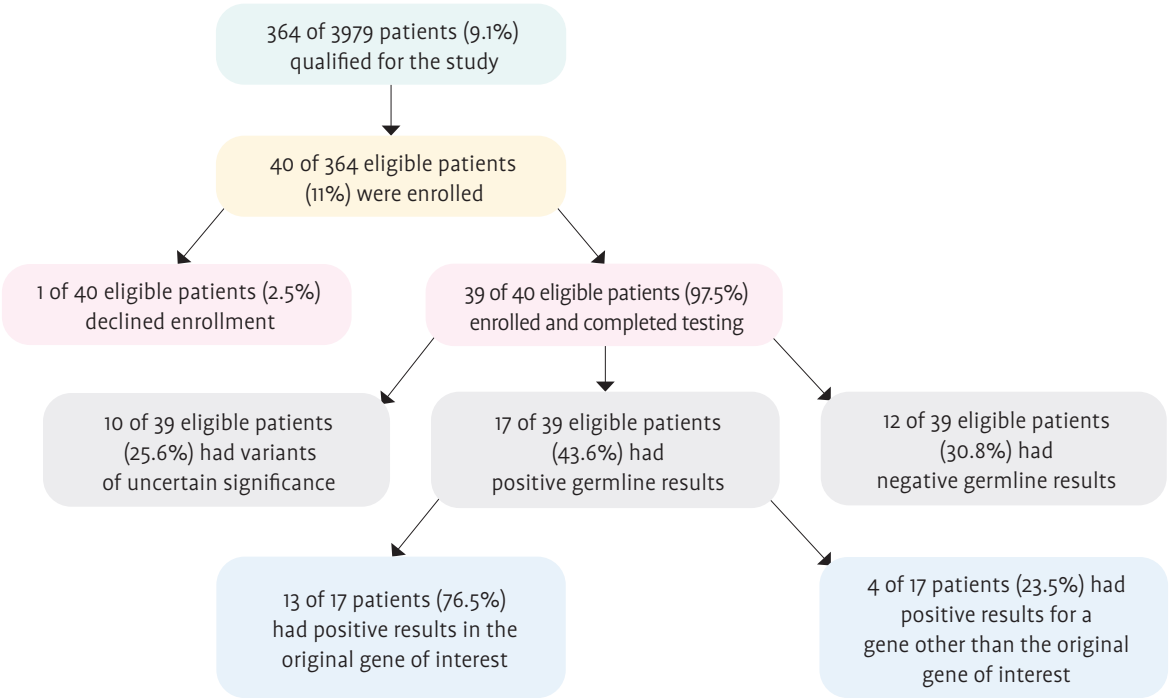

Table 2. Demographics of Patients Who Completed Testing, Grouped by Tumor Type[a]

Type of Cancer	Number of Patients (Positive, %)	Average Age (Positive Patients, year)	Number of males (Positive Patients, %)	Number of Females (Positive Patients, %)
Brain	5 (60%)	61 (65)	5 (60%)	0
Gastrointestinal	9 (67%)	73 (72)	4 (75%)	5 (60%)
Melanoma	4 (25%)	68 (49)	3 (33%)	1 (0%)
Gyn	11 (27%)	65 (62)	0	11 (27%)
Prostate	3 (33%)	69 (56)	3 (33%)	0
Other	7 (43%)	68 (78)	3 (66%)	4 (25%)

[a] *Positive* denotes patients who received results indicating likely pathogenic or pathogenic germline variants.

positive germline results defined as 1 or more pathogenic or likely pathogenic variants. (These 17 patients represented 43.6% of 39 eligible patients, although they all did not have mutations in the originally identified genes, and some had co-occurring variants of uncertain significance). Further, of the 39 eligible patients, 10 (25.6%) had 1 or more variants of uncertain significance only (25.6%), and 12 (30.8%) had negative testing. Of the patients with positive results, 13 of the 17 (76.5%) tested positive for mutations in the originally identified gene, whereas 4 of the 17 (23.5%) had positive results but not for the suspected gene based on the results of tumor testing only.

To increase the utility of genetic testing for patients with cancer, other barriers (eg, a lack of referrals to genetic counseling by treating oncologists) must be overcome.

The tumor types of patients who enrolled with demographic information provided are listed in **Table 2**. Overall, our study enrolled more patients with tier-1 mutations in gynecologic cancers; however, this tumor type had a relatively low rate of germline confirmation (27%) compared with other tumor types. Patients with GI cancers or brain cancers that displayed a tier-1 mutation had the highest rate of germline confirmation (67% and 60%, respectively). The average age of enrolled patients with GI or brain cancers did not differ significantly between those with confirmed germline mutations and those that were somatic mutations only.

Gene variants found in patients who were enrolled and who completed germline genetic testing were pathogenic or likely pathogenic, variants of uncertain significance (VUS), or not of germline origin (somatic-only). **Table 3** shows identified germline and somatic-only variants in our cohort. Of note, some patients were positive for more than 1 variant. Although a few patients carried more than 1 pathogenic or likely pathogenic variant, most had a combination of 1 pathogenic or likely pathogenic variant with co-occurring variants of uncertain significance.

Variant allele frequency (VAF) of 40% to 60% of a deleterious allele in known germline predisposition genes is used to identify individuals likely to have a pathogenic germline variant.[28] Patients who had a tier-1 variant that was confirmed to be positive in the germline had an average variant allele frequency of 48% on the somatic panel with the lowest observed being 20% and the highest observed being 78%.

Discussion

Somatic mutational profiling has increased in prevalence and has become the standard of care for many cancers. Somatic tumor testing is primarily used to identify potential alterations that could be targeted with specific drugs, but we emphasize that it has supplemental utility when used to investigate variants known to have a higher likelihood of being germline in origin. Identifying pathogenic variants of germline origin is important for optimizing care for patients with cancer, and it offers an added benefit of qualifying at-risk family members for cascade testing.[29]

The number of patients who had somatic profiling and then were enrolled in germline testing was lower than in previous similar studies. However, our cohort was limited by the fact that our institute is a private cancer center with more limited access to large patient populations. To increase the utility of genetic testing for patients with cancer, other barriers (eg, a lack of referrals to genetic counseling by treating oncologists) must be overcome. Many patients who screened positive for a tier-1 mutation were not referred by their treating physicians due to a variety of factors including the treating physicians' own clinical assessment of germline risk or concerns about the privacy

(*Continued on page 73.*)

Table 3. Results of Germline Genetic Testing[a]

Test Result	Gene	Mutation
Pathogenic or likely pathogenic	***BRCA2***	**c.5946delT**
Pathogenic or likely pathogenic	***BRCA1***	**c.3749_3752del**
Pathogenic or likely pathogenic	***MUTYH***	**c.1187G>A**
Pathogenic or likely pathogenic	***BRCA1 and CHEK2***	**c. 68_69del, c. 1100del**
Pathogenic or likely pathogenic	***CDKN2A***	**c.146T>C**
Pathogenic or likely pathogenic	***BRCA1***	**c.68_69del**
Pathogenic or likely pathogenic	***FH***	**c.1431_1433dup**
Pathogenic or likely pathogenic	***ATM, NBN,*** *CASR*	**c.217_218del, c.9139C>T (pos mosaic),** c.1190G>A (pos mosaic)
Pathogenic or likely pathogenic	***BRCA2, MUTYH***	**c.1456C>T, c.1187G>A**
Pathogenic or likely pathogenic	***MUTYH***	**c.536A>G**
Pathogenic or likely pathogenic	***BRCA2***	**c.9253del**
Pathogenic or likely pathogenic	***CHEK2****, CDKN2A, PDGFRA*	**c.1100del**, c.9_32del, c.1367G>A
Pathogenic or likely pathogenic	***CHEK2***	**c.1100del**
Pathogenic or likely pathogenic	***BRCA2***	**c.5946del**
Pathogenic or likely pathogenic	***PMS2,*** *DIS3L2, MSH3, PRKAR1A*	**c.1A>T,** c.943G>T, c.886C>T, c.155A>G
Pathogenic or likely pathogenic	***BRCA2***	**c.3336del**
Pathogenic or likely pathogenic	***CFTR,*** *NBN*	**c.1210-34TG[11]T[5],** c.442A>G
Variant of uncertain significance	*CDKN1C, FLCN, NTHL1*	c.173A>G, c.176G>A, c.607G>A
Variant of uncertain significance	*APC*	c.829G>T
Variant of uncertain significance	*RUNX1*	c.442A>G
Variant of uncertain significance	*DICER1*	c.493T>C
Variant of uncertain significance	*NBN*	c.1848A>G (silent)
Variant of uncertain significance	*PALB2, PTCH1, TSC2*	c.371C>A, c.49_51dup, c.4069A>C
Variant of uncertain significance	*POLE*	c.1470del
Variant of uncertain significance	*CASR, SDHA*	c.844G>A, c.5C>T
Variant of uncertain significance	*MSH3*	c.3188T>C
Variant of uncertain significance	*BLM, WRN*	c.968A>G, c.4099_4100delinsCA
Not of germline origin	*DICER1*	c.904-1G>A
Not of germline origin	*BRCA1*	c.5213_5215delGAG
Not of germline origin	*PTEN*	c.511C>T
Not of germline origin	*BAP1*	c.1761delA
Not of germline origin	*BRIP1*	c.3682G>T
Not of germline origin	*BAP1*	c.189dupT
Not of germline origin	*BRIP1*	c.1438dupA
Not of germline origin	*BRCA2*	c.2020G>T
Not of germline origin	*BRCA1, ATM, BAP1*	c.1016delA, c.1071dupT, c.1050delC
Not of germline origin	*ATM*	c.2413C>T
Not of germline origin	*MSH6*	c.3261delC
Not of germline origin	*BRIP1*	c.821delC

[a] Variants shown in bold are pathogenic/likely pathogenic; variants in roman are variants of uncertain significance.

(*Continued from page 71.*)
of genetic information. Other factors, including the COVID-19 pandemic, may have affected participation in the study, as it has a significant impact on clinical research and oncology management (eg, enrollment in studies, continuity of treatments).[30] Further, outcomes of other studies have shown that a majority of patients remain concerned about the implications of genetic testing, particularly as they relate to incidental findings, information overload, and a perceived lack of clear benefit from results.[31] Patient education and concerted efforts to clarify the benefits of germline testing can reduce some of these barriers.

> Our findings support expanding clinical criteria and lowering barriers for germline genetic testing in patients with cancer

Importantly, the tier system was not based on gene penetrance or degree of cancer risk. Rather, it was based on the probability that variants found in a particular gene via somatic testing could be of germline origin as noted in previous studies.[3–7] For example, somatic mutations in *TP53* are not often found to be germline in origin even though it is a highly penetrant gene with a high risk of cancer. Conversely, monoallelic mutations in *MUTYH* confer little to no increased risk for cancer; however, it is common to be a carrier of a *MUTYH* mutation and, when it is detected via somatic testing, the mutation commonly has a germline origin. Our study identified common founder mutations in genes associated with hereditary cancer syndromes (eg, *BRCA1*, *BRCA2*, *CHEK2*, and *MUTYH*).[32] We observed a 44% confirmatory rate for patients with suspected high-risk germline mutations, which confirms that our approach can identify patients carrying mutations in cancer predisposition genes from their somatic profiling results. However, mutations in our high-risk gene list (tier 1) were only found in 9.1% of overall somatic profiling results; this was well under the estimated 15% of cancers attributable to hereditary predisposition. Further, we identified more than 1 variant in some patients with few pathogenic or likely pathogenic variants in more than 1 gene; a majority of patients had a pathogenic or likely pathogenic variant with 1 or more co-occurring variants of uncertain significance. This indicates that benefit can be gained from our risk stratification approach, but the current screening mechanism to identify patients with hereditary cancers is incomplete.

Prior reports have estimated that variants with a Variant allele frequency of between 40% to 60% should be suspected as being of germline origin and that they merit confirmatory germline testing.[33] Our enrolled patients who were positive for pathogenic or likely pathogenic variants of germline status had an average Variant allele frequency of 48%. Still, we observed a wide distribution of variant allele frequency on somatic panels that were later confirmed as germline variants. Therefore, scrutiny of variant allele frequency alone on somatic tests is not a reliable way to differentiate somatic from germline variants. These findings are comparable to conclusions drawn by investigators in other studies.[10] Integrating somatic and germline testing along with other clinical information (eg, personal and family history) will be essential for achieving a high identification rate of hereditary cancers in the current clinical environment.

Conclusion

Implementing a somatic profile–based screening program to identify inherited cancer predisposition syndromes is feasible in patients who may not meet current germline genetic testing guidelines. Our study showed about 11% of patients with high-risk (tier-1) mutations underwent genetic counseling and testing, and an inherited predisposition to cancer was confirmed in about 44% of these patients—which is in line with positive detection rates from tumor-only genomic profiling of about 4% that was reported in previous studies.[12]

Germline genetic evaluation remains underused even when payer constraints are removed as an obstacle. Barriers such as patient education and lack of referrals by oncologists must be addressed to maximize the utility of genetic testing for patients with genetic predispositions to cancer. The presence of a mutation in a high-risk (tier-1) cancer predisposition gene identified through somatic profiling requires confirmatory germline testing. Our findings support expanding clinical criteria and lowering barriers for germline genetic testing in patients with cancer. OI

Sourat Darabi, MS, PhD, is the director of Precision Oncology for Hoag's Center for Applied Genomic Technologies at the Hoag Family Cancer Institute in Newport Beach, California. Jeanne P. Homer, MS, is a genetic counselor and supervisor of the hereditary cancer program at Hoag Family Cancer Institute in Newport Beach, California. Chelsey B. Torres, MS, is a genetic counselor at the hereditary cancer program at Hoag Family Cancer Institute in Newport Beach, California. Carlos E. Zuazo, MSc, is the translational research scientist for Hoag's Center for Applied Genomic Technologies at the Hoag Family Cancer Institute in Newport Beach, California. Valentina Dalili-Shoaie, MD, FACMG, is a medical geneticist; Michael J. Demeure, MD, MBA, is the medical director of Hoag's Precision Medicine Program; and David R. Braxton, MD, is the director of Molecular Pathology at the Hoag Family Cancer Institute in Newport Beach, California. Demeure is also an adjunct professor at the Translational Genomics Research Institute in Phoenix, Arizona.

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