Discrepancies between tumor genomic profiling and germline genetic testing

K. Pauley1*, C. Koptiuch1, S. Greenberg1, W. Kohlmann1, J. Jeter2, S. Colonna2, T. Werner2, C. Kinsey2, G. Gilcrease2, J. Weis2, J. Whisenant3, V. Florou2 & I. Garrido-Laguna2

1Family Cancer Assessment Clinic, Huntsman Cancer Institute, Salt Lake City; 2Department of Internal Medicine, Huntsman Cancer Institute, Salt Lake City; 3Department of Medical Oncology and Hematology, Utah Cancer Specialists, Salt Lake City, USA

Available online 1 July 2022

Background: Tumor genomic profiling (TGP) often incidentally identifies germline pathogenic variants (PVs) associated with cancer predisposition syndromes. Methods used by somatic testing laboratories, including germline analysis, differ from designated germline laboratories that have optimized the identification of germline PVs. This study evaluated discrepancies between somatic and germline testing results, and their impact on patients.

Patients and methods: Chart reviews were carried out at a single institution for patients who had both somatic and designated germline genetic testing. Cases with discrepant results in which germline PVs were not detected by the somatic laboratory or in which variant classification differed are summarized.

Results: TGP was carried out on 2811 cancer patients, 600 of whom also underwent designated germline genetic testing. Germline PVs were identified for 109 individuals. Discrepancies between germline genetic testing and tumor profiling reports were identified in 20 cases, including 14 PVs identified by designated germline genetic testing laboratories that were not reported by the somatic laboratory or in which variant classification differed are summarized. Three PVs identified by designated germline laboratories are targets for poly adenosine diphosphate-ribose polymerase (PARP) inhibitors and resulted in different treatment options. Of the PVs identified by designated germline laboratories, 60% (n = 12) were in genes with established associations to the patient’s cancer, and 40% of the PVs were incidental. The majority (90%) of all discrepant findings, both contributory and incidental, changed management recommendations for these patients, highlighting the importance of comprehensive germline assessment.

Conclusions: Methods used by somatic laboratories, regardless of the inclusion of germline analysis, differ from those of designated germline laboratories for identifying germline PVs. Unrecognized germline PVs may harm patients by missing hereditary syndromes and targeted therapy opportunities (e.g. anti-programmed cell death protein 1 immunotherapy, PARP inhibitors). Clinicians should refer patients who meet the criteria for genetic evaluation regardless of somatic testing outcomes.

Key words: hereditary cancer, somatic testing, germline genetic testing, precision medicine

INTRODUCTION

The use of tumor genomic profiling (TGP) in oncology is rapidly increasing. TGP may inform a patient’s prognosis, selection of targeted therapies, and clinical trial options. TGP, which profiles genetic alterations within tumors, reveals variants that may be isolated to a patient’s cancer cells (i.e. somatic variants) or present in every cell of the patient’s body (i.e. germline variants).1,2 TGP is distinct from germline genetic testing that sequences a person’s DNA to identify genetic variants associated with hereditary cancer predisposition syndromes.3 As a result, utilizing both TGP and germline genetic testing when appropriate yields the highest likelihood of finding clinically actionable results that could impact patient care and familial cancer screenings.4 TGP may not assess for underlying hereditary cancer predisposition syndromes.5 While TGP may incidentally identify germline pathogenic or likely pathogenic variants (PVs) associated with hereditary cancer risk, testing processes used by somatic laboratories differ from those utilized by designated germline laboratories.5,6 Differing variant classification methods, intentional exclusion of
germline variants on TGP reports, low variant allele fraction, and allelic dropout all may lead to discrepancies between PVs found by TGP and designated germline genetic testing.\(^7\)-\(^9\)

Designated germline genetic testing laboratories use technologies optimized for the identification of germline PVs, including those that may be challenging to detect.\(^10\)-\(^12\) Additionally, these laboratories classify variants based on guidelines published by the American College of Medical Genetics and Genomics which differ from those guidelines utilized by TGP laboratories.\(^13\),\(^14\) The main difference between these sets of guidelines is which lines of evidence are considered most important for classification of variants.\(^8\)

Germline genetic testing may identify variants with clinical utility that are not detected by somatic testing laboratories. The identification of germline PVs is critical in clarifying a patient’s future cancer risk and possibly introducing new treatment options such as poly adenosine diphosphate-ribose polymerase (PARP) inhibitors or anti-programmed cell death protein 1 (PD-1) immunotherapy options.\(^2\),\(^4\),\(^15\)-\(^18\) Germline genetic testing is also valuable for family members and can initiate cascade testing for relatives, a process which remains underutilized, with a recent study showing only a 16% uptake of family testing in a cohort of colorectal cancer patients.\(^19\)

There are many reasons why germline PVs may not be recognized by somatic testing laboratories. These laboratories specialize in identifying somatic mutations of therapeutic relevance; the primary purpose of TGP is not to identify germline mutations.\(^3\) Some laboratories exclude germline variants from their final reports in an attempt to report on only somatic alterations arising in the tumor. While germline PVs may be identified by these laboratories, they are not included in the clinical report.\(^4\) Other laboratories that include germline findings in their report may still underreport PVs if there is no matched sample for germline analysis. Additionally, TGP gene panels do not include all genes relating to hereditary cancer predisposition syndromes and their analysis methods may not include full gene sequencing or comprehensive deletion/duplication analysis. Lower depth of sequencing reads may also contribute to germline PVs not being reported by somatic testing laboratories.\(^2\),\(^4\),\(^9\),\(^20\) In order to improve specificity, some somatic testing laboratories exclude germline and intronic variants from analysis.\(^21\)

There are a number of limitations to using TGP alone to assess for germline PVs which include lower sensitivity and quality of variant classification of somatic laboratories compared to designated germline laboratories. Overlooked germline PVs may give oncologists false reassurance of the absence of a hereditary syndrome and deprive patients and their families of targeted therapy opportunities (e.g. anti-PD1 immunotherapy, PARP inhibitors), cancer risk clarification, and tailored medical management options.\(^1\),\(^3\),\(^8\)

Utilizing both TGP and germline genetic testing in appropriate cases may provide the highest yield of findings that could affect patient care. Many oncology practice guidelines have incorporated recommendations for germline testing when a PV with possible clinical implications of germline is identified by TGP.\(^22\)-\(^25\) Germline analysis following TGP may identify PVs in patients who would not otherwise meet the criteria for germline testing alone.\(^4\)

This study aimed to identify discrepancies between PVs reported by somatic testing laboratories and designated germline laboratories. The goal of this study is to evaluate the utility of somatic testing as an equivalent proxy for germline genetic testing.

**METHODS**

An institutional retrospective chart review of patients from a single institution with both TGP and germline genetic testing data available was carried out. All patients who underwent TGP tests ordered by clinicians at the Huntsman Cancer Institute through two commercial laboratories between 1 January 2014 and 31 December 2019 were queried. The specific tumor-inclusive panels ordered varied by clinician discretion. Information regarding which laboratory was utilized, cancer type, sample type, and collection date was collected with this dataset. Patient identifiers (e.g. name, date of birth, and medical record number) were also collected.

A subset of patients for whom TGP was ordered were also referred for genetic counseling and testing at the discretion of their oncologists due to their personal and/or family history. Those with PV discovered through designated, Clinical Laboratory Improvement Amendments (CLIA)-certified germline genetic testing laboratories (e.g. Ambry, City of Hope, Invitae, Myriad) were analyzed for this study. Test reports from designated germline laboratories were compared to results from TGP via chart review.

Current versions of the National Comprehensive Cancer Network (NCCN) Genetic/Familial High-Risk Assessment guidelines\(^22\),\(^23\) were used to determine whether the patients’ personal and/or family history currently meet clinical testing criteria for genetic testing. These guidelines were also used to inform which genes have an established association with and management recommendations for the individuals’ cancer type. Germline genetic testing was ordered based on NCCN criteria and genetic counselor discretion at the time of the initial consultation.

Variants with interpretations of likely pathogenic (LP) or pathogenic (P) that had multiple submitters and were reviewed by the expert panel on ClinVar were accepted and used to assess variant classification discrepancies. ClinVar is a publicly available database of the relationship between genotype and phenotype as reported by various laboratories and research initiatives. ClinVar follows the American College of Medical Genetics recommendations for variant classification.\(^11\) The gene HOXB13 is classified as an increased-risk allele which is considered a PV in this study.

Gene panel information and results of TGP and germline testing were obtained from the clinical test reports. Chart review of patients who had TGP and germline testing was
carried out to obtain information on treatment, demographic data, and outcomes. This study was approved by the University of Utah Institutional Review Board.

RESULTS

Description of somatic and germline test utilization
A total of 2811 individuals at the Huntsman Cancer Institute underwent TGP through two somatic testing laboratories. Within this cohort of patients whose tumor was subjected to TGP, 21.3% (n = 600) also independently had germline genetic testing ordered from a designated germline laboratory as part of their clinical care at the discretion of their clinician. Germline PVs were identified in 18.2% (n = 109) of these 600 patients.

Study population
A comparison of germline testing with an identified PV and the corresponding TGP report revealed discrepancies in 20 cases (18.3%). For this study, discrepant cases are defined as PVs identified by designated germline laboratories but not reported by somatic laboratories, or as variants assigned a different classification, and PVs identified in a cancer predisposition gene not included in the somatic panel (Figure 1).

An additional two discrepant cases were found incidentally during chart review. In these cases, germline reports described variants classified as variants of uncertain significance (VUS) by the designated germline laboratories and ClinVar but reported as pathogenic by the somatic testing laboratories. Both patients also had other PVs identified by both the germline and somatic laboratories. Based on the methods utilized for this study, other cases similar to these may have been missed. Therefore, these cases have been excluded from this study because this situation could not be assessed for the whole cohort.

Description of cohort with discrepant results (n = 20)
The patients in this cohort were predominantly male (65%, n = 13). They had a variety of cancer diagnoses, with prostate cancer (15%, n = 3), pancreatic cancer (15%, n = 3), and colon cancer (15%, n = 3) being most prevalent. The median age of initial cancer diagnosis was 48 years (range: 7-72 years). All 20 patients met current NCCN criteria for genetic testing based on personal and/or family history noted in the medical record.22,23 Of these patients, 10 (50%) underwent germline genetic testing before their TGP results, 9 (45%) underwent TGP before their germline testing, and 1 patient had both germline genetic testing between their two tumor samples being sent for TGP. A variety of germline and TGP testing panels were utilized. These panels varied due to clinician discretion, sample type available, and assay options available at the time ordered. The majority (85%, n = 17) of TGP tests only analyzed somatic variants, while 15% (n = 3) utilized paired germline and somatic analysis by sequencing the patient’s tumor DNA and a matched normal blood/saliva sample in order to detect cell-free DNA (cfDNA) in blood specimens (Table 1).

Discrepant cases
Of the 20 discrepant cases identified, 14 PVs were reported by designated germline genetic testing laboratories but not by somatic testing laboratories. These PVs were identified in nine different genes (ATM, BRCA1, BRCA2, HOXB13, MEN1, MLH1, 9 RAD51D, PMS2, and SDHB). Six PVs were not detected because those specific genes were not analyzed by the somatic testing laboratory. The other eight unreported germline PVs were in genes analyzed by the somatic test.

Table 1. Demographic information of 20 patients with discrepant results between tumor genomic profiling and designated germline genetic testing reports

| Characteristic                        | n  | %   |
|--------------------------------------|----|-----|
| **Sex**                              |    |     |
| Female                               | 7  | 35  |
| Male                                 | 13 | 65  |
| **Indication**                       |    |     |
| Pancreatic cancer                    | 3  | 15  |
| Prostate cancer                      | 3  | 15  |
| Colon cancer                         | 3  | 15  |
| Other                                | 11 | 55  |
| **Age at first cancer diagnosis (years)** |   |     |
| 0-19                                 | 3  | 15  |
| 20-29                                | 1  | 5   |
| 30-39                                | 2  | 10  |
| 40-49                                | 4  | 20  |
| 50-59                                | 3  | 15  |
| 60-69                                | 5  | 25  |
| 70-79                                | 2  | 10  |
| **Timing of germline testing**       |    |     |
| Prior                                | 10 | 50  |
| After                                | 9  | 45  |
| Simultaneous                         | 1  | 5   |
| **Somatic testing type**             |    |     |
| Somatic only                         | 17 | 85  |
| Paired germline and somatic          | 3  | 15  |
Somatic testing laboratories utilized in this study provide limited technical information about their testing platforms. The reports did not provide enough technical information to determine why each of these variants were not identified. Possible reasons for exclusion include deeply intronic variants being outside of the detection range of the platform, somatic and germline alterations not being differentiated on reports, poor coverage in certain areas, and filtering out of germline PV to improve somatic variant sensitivity.

The remaining six discrepant cases were due to differing classifications given by designated germline and somatic testing laboratories. These variants were classified as pathogenic by germline laboratories and ClinVar but as VUS by somatic testing laboratories (Table 2). Since somatic testing platforms are not germline-validated tests, they do not result in amended reports when variants are reclassified.

Based on current NCCN guidelines, 12 of the 20 germline PVs (60%) are in genes associated with the patient’s diagnosis. The other eight PVs are considered secondary findings as these genes are unrelated to the diagnostic indication. Of the 20 PVs identified by designated germline laboratories, 90% (n = 18) resulted in new management recommendations based on NCCN guidelines from 202022,23 (Table 3).

### Clinical management

In three cases, the PVs not detected through TGP were in the genes BRCA1 (n = 2) and BRCA2 (n = 1) for which PARP inhibitors can be used. These three individuals had been diagnosed with ovarian cancer, pancreatic adenocarcinoma, and cholangiocarcinoma. PARP inhibitors are currently approved by the United States Food and Drug Administration (FDA) for patients with ovarian (ninarparib, olaparib, and rucaparib) and pancreatic cancer (olaparib). The identification of a BRCA1/2 PV by germline report changed treatment for two of the three patients. The third patient passed away after the completion of a clinical trial and before starting a PARP inhibitor.

The first patient whose germline genetic testing identified a BRCA1 PV was initially diagnosed with stage IIIC ovarian cancer in 2010. She underwent BRCA1/2 testing through Myriad Genetics in 2013 after she was found to have recurrent disease. Upon germline testing results and following two lines of therapy for recurrent disease (carboplatin/gemcitabine, and liposomal doxorubicin), she started treatment with olaparib. On disease progression 5 months later, TGP did not detect her germline BRCA1 mutation.

The second patient, whose germline genetic testing identified a PV in BRCA2, was diagnosed with metastatic pancreatic cancer in August 2018. He underwent germline genetic testing which analyzed 15 genes through Invitae Laboratory in 2018. He started therapy with gemcitabine and paclitaxel protein-bound, with progressive disease after two cycles. After identification of a BRCA2 PV, his treatment was switched to olaparib on which he had stable disease for ~9 months. The TGP report, issued in March 2019, did not identify the BRCA2 PV.

The final patient carries a germline PV in BRCA1. This patient was diagnosed with unresectable cholangiocarcinoma in January of 2019. Her germline genetic testing was reported in May 2019 while she was enrolled in a clinical trial of gemcitabine, cisplatin, and pegPH20, an investigational cancer drug. She then underwent radiation concurrently with capcitabine followed by

---

**Table 2. Summary data for PVs identified in this study**

| Gene   | Variant                        | TGP classification | Germline classification | Inclusion on TGP panel |
|--------|--------------------------------|--------------------|-------------------------|------------------------|
| ATM    | c.5763-1050A>G                 | Not identified     | PV                      | Yes                    |
| ATM    | c.5100del (p.Lys1701Serfs'13)  | Not identified     | PV                      | Yes                    |
| ATM    | c.8418+5_8418+8delGTGA (intron) | Not identified     | PV                      | Yes                    |
| BRCA1* | c.213-11T>G                    | Not identified     | PV                      | Yes                    |
| BRCA1  | c.975_910del (p.Pro2992_Thr3033del) | Not identified     | PV                      | Yes                    |
| CDKN2A | c.335_337dupGTC (p.Arg112_Leu113insArg) | VUS                | PV                      | Yes                    |
| CHEK2  | c.349A>G (p.Arg117Gly)         | VUS                | LPV                     | Yes                    |
| HBB    | c.251G>A (p.Gly84Glu)          | Not identified     | PV                      | No                     |
| MCM11  | c.784-9G>A (intron)            | Not identified     | PV                      | Yes                    |
| MLH1   | c.1990>G>A (p.Gly67Arg)        | VUS                | PV                      | Yes                    |
| MLH1   | c.1731G>A (p.Ser577->C)        | Not identified     | PV                      | Yes                    |
| MLH1   | c.1731G>A (p.Ser577->T)        | Not identified     | PV                      | Yes                    |
| MLH1   | c.2194A>T (p.Lys732')          | Not identified     | PV                      | No                     |
| PMS1   | c.137G>T (Ser46Ile) homozygous | Not identified     | PV                      | Yes                    |
| RAD51D | c.564_568delinsA (p.Val189Profs'4) | Not identified     | PV                      | No                     |
| SDHB   | c.287-1G>C (splice acceptor)   | Not identified     | PV                      | No                     |
| TP53   | c.1040G>A (p.Ala347Asp)        | VUS                | PV                      | Yes                    |
| TP53   | c.1040G>A (p.Ala347Asp)        | VUS                | PV                      | Yes                    |

Description of variant classification by TGP laboratories and germline genetic testing laboratories. PARP, poly adenosine diphosphate-ribose polymerase; PV, pathogenic variant; TGP, tumor genomic profiling; VUS, variant of uncertain significance.

*Eligible for PARP inhibitor.
FOLFIRINOX (5-fluorouracil, oxaliplatin, irinotecan). This patient underwent TGP twice, once before her germline genetic testing and once after. Both TGP samples were sent through the same laboratory. Both paired tumor and blood sample testing and cfDNA analysis were carried out. Neither testing identified the BRCA1 deletion. Her clinicians considered PARP inhibitor during her treatment course; however, the patient’s functional status declined and she eventually pursued palliative care.

Immune checkpoint inhibitors have emerged in recent years and are an important advancement in cancer therapeutics. In 2017, the FDA granted approval to pembrolizumab, a PD-1 inhibitor, to be used for patients with microsatellite instability-high (MSI-H) or mismatch repair-deficient (dMMR) solid tumors who have had tumor progression following prior treatments or no other viable options.26 In this study’s cohort, five patients received pembrolizumab. The majority (80%, n = 4) of these patients had mutations in the gene MLH1. The fifth patient had homozygous PMS2 PV resulting in a diagnosis of constitutional mismatch repair deficiency syndrome (CMMRD).

Among these five patients, one patient with metastatic appendiceal cancer (pseudomyxoma peritonei) had TGP in 2017 which identified a truncation variant in MLH1 and her tumor was MSI-H. While the somatic laboratory classified this MLH1 variant as a VUS, germline genetic testing through a germline-specific laboratory in February 2018 classified this as pathogenic. After surgical resection, the patient received initially 12 cycles of FOLFOX (5-fluorouracil and oxaliplatin) with no evidence of disease on completion. Three years later, metastatic disease to the liver was found and the patient received four cycles of FOLFIRI (5-fluorouracil, irinotecan) and bevacizumab with progressive disease. Upon identification of the MLH1 PV, pembrolizumab was initiated resulting in stable disease for 7 months. Treatment was changed to nivolumab/ipilimumab on which patient had stable disease for 4 months.

One patient with metastatic adrenal carcinoma underwent TGP in June 2017 and the MSI could not be determined. His 28-gene Invitae germline genetic testing panel identified a PV in the gene MLH1 in September 2017. After systemic chemotherapy with pemetrexed/cisplatin, cisplatin/methotrexate, and bilateral adrenalectomies, the patient started treatment with pembrolizumab when recurrent disease was identified. The patient remains on pembrolizumab 2 years later with almost complete resolution of disease.

Another patient with metastatic rectal cancer was initially treated with FOLFIRX in combination with pembrolizumab in the context of a clinical trial. He had favorable response and thus he eventually transitioned to maintenance 5-fluorouracil/pembrolizumab after nine cycles. After 6 months on maintenance therapy, the patient’s disease progressed, and his treatment was changed to FOLFIRI. TGP demonstrated the tumor was microsatellite stable but germline genetic testing identified a familial PV in the gene MLH1.

Both of the final two patients underwent TGP before starting pembrolizumab. Neither report included a comment on MSI. One patient’s PMS2 PV had previously been identified while the other patient’s MLH1 PV was identified 2 months after starting her treatment. The first patient had metastatic colorectal cancer treated initially with chemotherapy (FOLFOX/bevacizumab) followed by pembrolizumab. He received eight cycles with partial response of his disease before being lost to follow-up. The second patient had metastatic cholangiocarcinoma and started pembrolizumab. Unfortunately, this patient’s condition declined after only one cycle and she transitioned to comfort care.

DISCUSSION

The results of this study demonstrate several types of discrepancies between TGP and designated germline genetic testing. Almost one in five germline PVs in this cohort would not have been detected without referral for genetic counseling and testing. Germline PVs may have treatment implications, future cancer risks associated, and medical management changes for both the patient and their family members. Both germline genetic testing and TGP offer valuable information to clinicians and patients and utilization of both TGP and germline genetic testing, when appropriate, is necessary.
One important finding from this study is that 30% of the discrepant cases identified in this cohort were due to differing variant classifications. TGP and germline genetic testing laboratories utilize differing methods to classify variants.\textsuperscript{13,14} These guidelines give differing weight to various lines of evidence used when classifying a variant as uncertain, benign, or pathogenic.\textsuperscript{8,13,14} Additionally, many somatic tests are not considered validated assays for hereditary risk assessment and, therefore, amended reports for variant reclassifications are not issued. The American Society of Clinical Oncology recommends that TGP reports with possible germline PV be communicated to patients and confirmed by germline analysis.\textsuperscript{1} In this cohort, six variants had differing classifications issued and therefore may not have been flagged by physicians as important to discuss with patients.

In addition to these six variants which were called PV by germline laboratories but VUS by somatic laboratories, two variants were incidentally identified which were classified as PV by TGP and VUS by germline laboratories. Given the methods of this study which included only analyzing germline genetic testing with positive results, more findings of this nature may have been missed. The number of cases similar to this and the implications of these differences have not yet been well characterized.

There are a number of factors that can lead to a germline PV not being reported on a TGP platform. Some laboratories filter out germline PV and intronic variants in order to improve specificity of somatic findings. Sensitivity of single-nucleotide variants, insertions and deletions, and copy number variants is not 100%, especially at lower variant allele frequencies.\textsuperscript{21} Deeply intronic PV may not be reported if they are outside of the detection range of the TGP platform. Amended reports after reclassification of VUS are not routinely released. Due to the limited technical information provided in the somatic reports, it is not always clear why germline PVs were not detected.

In this study, germline testing identified actionable genetic aberrations in PVs that were not detected by somatic testing. This led to treatment changes for the affected patients and impacted the care of patients’ relatives. Given that PARP inhibitors are approved for breast, ovarian, and pancreatic cancer patients with germline \textit{BRCA1/2} PVs and PD-1 inhibitors are approved for patients with dMMR tumors, it is critical that we identify patients likely to derive benefit from these therapies. As a result, comprehensive TGP and germline work-up for patients who meet the criteria is essential.

Many of the PVs identified by designated germline testing resulted in new screening recommendations based on NCCN guidelines. Having a germline PV in many of the genes identified warranted changes to medical management for patients and their families including both risk reducing options (e.g. risk reducing mastectomy, chemo-prevention) and increased cancer surveillance (e.g. additional colonoscopies, breast magnetic resonance imaging, esophagastroduodenoscopy, magnetic resonance cholangiopancreatography, blood work) depending on the PV. For the 10 individuals who underwent TGP testing before germline genetic testing, these results would not have been known and these patients and their family members would have missed cancer screening opportunities to reduce their risk of diagnosis of cancer at late stage. The majority of patients in this study harbored a germline PV that could provide a partial explanation for their diagnosis. Of the eight patients with incidental findings, five reported a family history of cancer associated with the PV identified as per pedigree information collected by their genetic counselor at the time of initial appointment. This finding highlights the importance of collecting a thorough family history and multigene panel tests. If a larger, more comprehensive panel had not been ordered, many of these PVs would not have been detected.

The identification of a hereditary cancer predisposition syndrome is important for family members as they may also have increased cancer risks. Germline genetic testing is important in these families to determine who needs additional cancer screening. Another benefit of referring appropriate patients to genetic counselors for germline genetic testing is the current shift from site-specific testing to multigene germline panel testing occurring in the field.\textsuperscript{27} While site-specific or gene-specific testing may still be appropriate in some cases, many arguments can be made for the utility of a multigene panel genetic test. In this cohort of patients, five incidental PVs in genes for which clinical management changes are recommended would have been undetected without the use of multigene panel testing. Additionally, germline laboratories are dedicated to variant reclassification and notification of patients with these updates.\textsuperscript{13,28} As of January 2021, as per the report from the somatic testing laboratory representatives, TGP laboratories do not provide reclassifications over time.

\textbf{Study limitations and future research directions}

This was a retrospective study conducted at a single institution. The identification of two incidental discrepant findings highlights the possibility that other discrepancies may have been missed due to the methods utilized in this study. A future study could have modified methods in order to identify all such cases. A prospective study of everyone who undergoes TGP, germline testing, or both may capture the whole group and provide a more accurate picture of the discrepancies between the two.

Another limitation of this study is that it was not always clear in this study why PVs were not identified, and whether it was due to factors such as genomic loss within the tumor or technology barriers. Lastly, since beginning work on this project, a number of collaborations between somatic and germline testing have arisen.\textsuperscript{29,30} It would be interesting to see if there is an improvement on the number of discrepancies reported as these collaborations move forward.

\textbf{Conclusions}

The methods used by somatic laboratories alone are inadequate to identify some germline PVs. Overlooked
germline PVs may miss identification of hereditary syndromes and targeted therapy opportunities (e.g. PARP inhibitors). Clinicians should refer their patients who meet the criteria for genetic evaluation to genetic counselors regardless of the results of their somatic testing. The identification of PV in genes not relating to the patient’s primary indication highlights the importance of collecting a thorough family history and a panel approach to germline genetic testing. Somatic testing was not found to be an equivalent proxy for germline genetic testing in this study. Integrating both TGP and designated germline testing into clinical practice may provide the highest yield for clinically actionable findings.

**FUNDING**

This work was supported by the Genetic Counseling and Research Informatics Shared Resources at Huntsman Cancer Institute at the University of Utah (no grant number), and by the National Cancer Institute of the National Institutes of Health [grant number P30CA042014]. The content is solely the responsibility of the authors and does not necessarily reflect the official views of the NIH.

**DISCLOSURE**

The authors have declared no conflicts of interest.

**REFERENCES**

1. Robson ME, Bradbury AR, Arun B, et al. American Society of Clinical Oncology policy statement update: genetic and genomic testing for cancer susceptibility. *J Clin Oncol.* 2015;33(31):3660-3667.

2. DeLeonardis K, Hogan L, Cannistra SA, Rangachari D, Tung N. When should tumor genomic profiling prompt consideration of germline testing? *J Oncol Pract.* 2019;15(9):465-473.

3. Jain R, Savage MJ, Forman AD, Mukherji R, Hall M. The relevance of hereditary cancer risks to precision oncology: what should providers consider when conducting tumor genomic profiling? *J Natl Compr Canc Netw.* 2016;14:795-806.

4. Lincoln SE, Nussbaum RL, Kurian AW, et al. Yield and utility of germline testing following tumor sequencing in patients with cancer. *JAMA Netw Open.* 2020;3(10):e2019452.

5. Mandelker D, Zhang L, Kemel Y, et al. Mutation detection in patients with advanced cancer by universal sequencing of cancer-related genes in tumor and normal DNA vs guideline-based germline testing. *JAMA.* 2017;318(9):825-835.

6. Meric-Bernstam F, Brusco L, Daniels M, et al. Incidental germline variant interpretation in 1000 advanced cancers on a prospective somatic genomic profiling protocol. *Ann Oncol.* 2016;27(5):795-800.

7. Raymond VM, Gray SW, Roychowdhury S, et al. Germline findings in tumor-only sequencing: points to consider for clinicians and laboratories. *J Natl Cancer Inst.* 2015;108(4).

8. Moody EW, Vagher J, Espelin W, Goldgar D, Hagerty KJ, Gammon A. Comparison of somatic and germline variant interpretation in hereditary cancer genes. *JCO Precis Oncol.* 2019;3:1-8.

9. Forman A, Sotelo J. Tumor-based genetic testing and familial cancer risk. *Cold Spring Harb Perspect Med.* 2020;10(8).

10. Rhees J, Arnold M, Boland C. Inversions of exons 1-7 of the MSH2 gene is a frequent cause of unexplained Lynch syndrome in one local population. *Fam Cancer.* 2014;13:219-225.

11. Farber-Katz S, Huan V, Wu S, et al. Quantitative analysis of BRCA1 and BRCA2 germline splicing variants using a novel RNAmassively parallel sequencing assay. *Front Oncol.* 2018;8:286.

12. Karam R, Krempeky K, Richardson M, et al. RNA genetic testing in hereditary cancer improves variant classification and patient management. *Ambry Genetics Abstract Poster #167.* 2019.

13. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015;17(5):405-424.

14. Li MM, Datto M, Duncavage EJ, et al. Standards and guidelines for the interpretation and reporting of sequence variants in cancer: a joint consensus recommendation of the association for molecular pathology, American Society of Clinical Oncology, and College of American Pathologists. *J Mol Diagn.* 2017;19(1):4-23.

15. Audeh MW, Carmichael J, Pensom RT, et al. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer: a proof-of-concept trial. *Lancet Oncol.* 2010;3:9737;245-251.

16. Tutt A, Robson M, Garber JE, et al. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: a proof-of-concept trial. *Lancet Oncol.* 2010;3:9737;235-244.

17. Maby P, Tougeron D, Hamieh M, et al. Correlation between density of CDB+ T-cell infiltrate in microsatellite unstable colorectal cancers and frameshift mutations: a rationale for personalized immunotherapy. *Cancer Res.* 2015;75(17):3446-3455.

18. McCabe N, Turner N, Lord CJ, et al. Deficiency in the repair of DNA damage by homologous recombination and sensitivity to poly(ADP-ribose) polymerase inhibition. *Cancer Res.* 2006;66(16):8109-8115.

19. Uson PLS, Rieger-Johnson D, Boardman L, et al. Germline susceptibility gene testing in unselected patients with colorectal adenocarcinoma: a multicenter prospective study. *Clin Gastroenterol Hepatol.* 2020;22(3):e508-e528.

20. Mandelker D, Zhang L. The emerging significance of secondary germline testing in cancer genomics. *J Pathol.* 2018;244(5):610-615.

21. Finkle JD, Boulus H, Driessen TM, et al. Validation of a liquid biopsy assay with molecular and clinical profiling of circulating tumor DNA. *NPJ Precis Oncol.* 2021;5:63.

22. Daly MB, Pal T, Berry MP, et al. Genetic/familial high-risk assessment: breast, ovarian, and pancreatic, version 2.2021, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw.* 2021;19(1):77-102.

23. Gupta S, Weiss JM, Burke C, et al. NCCN Guidelines® insights: genetic/familial high-risk assessment: colorectal version 1.2021. *J Natl Compr Canc Netw.* 2021;19(10):1122-1132.

24. Mandelker D, Donoghue M, Talukdar S, et al. Germline-focused analysis of tumour-only sequencing: recommendations from the ESMO Precision Medicine Working Group. *Ann Oncol.* 2019;30(8):1212-131.

25. Li MM, Chao E, Esplin ED, et al. Points to consider for reporting of germline variation in patients undergoing tumor testing: a statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med.* 2020;22(7):1142-1148.

26. Administration US FDA. FDA grants accelerated approval to pembrolizumab for first tissue/site agnostic indication2017.

27. Thomas MH, Higgs L, Modesitt SC, Schroen AT, Ring KL, Dillon PM. Cases and evidence for panel testing in cancer genetics: is site-specific testing dead? *J Genet Couns.* 2019;28(3):700-707.

28. Mersch J, Brown N, Pirzadeh-Miller S, et al. Prevalence of variant reclassification following hereditary cancer genetic testing. *JAMA.* 2018;320(12):1266-1274.

29. Tempus. Tempus. Launches Hereditary Cancer Germline Exam, xG2021. 2019.

30. Foundation Medicine. Foundation Medicine and InformedDNA® Collaborate to Improve Access to Genetic Counseling for Advanced Cancer Patients. 2021.