

Graduate Thesis

Assessing Tumor Genomic Profiling Reports
for Genetic Counseling Referral Indications

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Submitted in partial completion of the Master of Science Degree
at Sarah Lawrence College, May 2016.

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Abstract

Our purpose is two-fold: (1) to identify patients who may benefit from referrals to cancer genetic counseling by characterizing the alterations reported in tumor profiling reports without matched germline control, and (2) to assess the utility of public database ClinVar in providing further information on these alterations. We assessed 160 reports across 66 tumor types. 127 (79%) reports had a mutation in 1 of 86 selected cancer predisposition genes. Of these, 29 (23%) did not meet ACMG/NSGC criteria for referral based on tumor type alone. 19% of mutations in selected genes were found in ClinVar, with 15% reported as both germline and on the pathogenic spectrum. 31% of VUSs in selected genes were found in ClinVar, with 2% reported as both germline and on the pathogenic spectrum. Our results highlight a potential to miss patients at increased risk of cancer predisposition syndromes based on tumor type alone.

Key terms

cancer genetics, cancer predisposition, genetic counseling, tumor profiling

Introduction

Problem Statement & Significance

In recent years, genomic profiling of tumors has become an increasingly common tool by which oncologists determine customized treatment options for patients (Zhao et al., 2015). Treatments are primarily designed to target somatic mutations – or mutations acquired after conception – but the technology also has the ability to detect germline mutations – or mutations present since conception. Despite significant advances in technology, it remains difficult with solely a tumor sample to differentiate between somatic and germline mutations. As a result, germline mutations detected by this testing methodology are often reported without differentiation. This poses a problem for patients and clinicians alike, as germline mutations have the potential to be associated with hereditary cancer predisposition syndromes, affecting both the patient and his or her family members. This study aims to characterize the significance of mutations observed in tumor profiling results and to describe instances where follow-up referrals for genetic counseling would be recommended based on tumor profiling results. This study also aims to explore the clinical utility of public database ClinVar in determining whether tumor profiling companies call mutations differently than other sources that perform germline testing.

Background & Context for Literature Review

Over 10,000 tumor genomes have been sequenced in research or clinical settings to date (Wang et al., 2015). Increased availability of tumor profiling data has enabled researchers and oncologists to detect patterns in tumor development, as well as relationships between tumor genotypes and responses to various drug treatments. However, the majority of this complex data has yet to be fully understood, and to date, tumor profiling research has

been treatment-oriented and focused largely on somatic alterations (Meric-Bernstam et al., 2016; Schrader et al., 2015). This study aims to expand our understanding of the importance of considering germline mutation risk detection and prevention in the setting of tumor profiling.

Literature Review

Genetic Heterogeneity of a Tumor

Part of the challenge in treating tumors is due to their heterogeneous genetic makeup. An individual tumor may have a few to hundreds of alterations and each of these alterations may be present in only a few to all of the cells throughout the tumor itself (Wang et al., 2015). For example, about half of all solid tumors studied have undergone a duplication event, resulting in the presence of aneuploidy in a percentage of these tumors' cells: the later the duplication event in the process of tumorigenesis, the smaller the percentage of cells with that aneuploidy (Wang et al., 2015).

Tumor Development and Technological Advancements

It takes time for a tumor to develop the genetic alterations needed to turn non-malignant cells into a malignant disease. In the earliest stages of tumorigenesis, there are fewer alterations present, and only one cell has notable mutations. This cell, according to Novell's theory of tumor development put forth in 1976, continues to replicate over time, producing "clones" (or cell populations) with varying amounts and degrees of mutations (Wang et al., 2013). These clones competitively replicate, until one of the clones gains a sufficient number of mutations to achieve a significantly faster replication rate than the others. As a result, this clone grows to dominate the overall tumor cell population and propel it toward a cancerous state. This theory has evolved through continued research, and it is now

suspected that a tumor may begin with multiple clones, further contributing to the tumor's genetic heterogeneity and complexity (Wang et al., 2013).

Tumorigenesis may be described in greater depth using colorectal cancer (CRC) as a model, due to its somewhat predictable course. CRC develops sequentially, from dysplasia of epithelium cells that progress to benign adenomas, to invasion of local tissues and metastasis (Fearhead, Wilding, & Bodmer, 2002). Genetically, CRC develops through the activation and inactivation of oncogenes and tumor suppressor genes, respectively. There are several main genes involved in this process: the *APC* tumor suppressor gene, the *RAS* oncogene, and the *TP53* gene involved in controlling the death of tumor cells (Knudson, 2001). An individual may be born with mutations (germline mutations) in these genes, thereby inheriting a cancer predisposition syndrome, and/or acquire mutations (somatic mutations) throughout his or her life. From genetic studies of serial biopsies, it is possible to distinguish the stages of CRC development through the characteristic somatic mutations that occur in each stage. For example, somatic mutations in the *APC* gene tend to occur in early stages while *TP53* mutations tend to occur later (De la Chapelle, 2004). A five-hit hypothesis of the genetic basis of colorectal tumor development has been proposed consisting of two *APC* gene mutations (presumably one in each copy of the gene), followed by one *RAS* gene mutation and finally two *TP53* mutations (one in each copy of the gene) (Knudson, 2001).

Driver Mutations Versus Passenger Mutations

Some tumor mutations are more aggressive than others. Vogelstein et al. (2013) describes driver mutations as mutations that “confer a selective growth advantage to the tumor cell” and passenger mutations as ones that have “no direct or indirect effect on the selective growth advantage of the cell” (p. 1548-1549). It is the driver mutations that speed

the tumor to develop at a faster rate and truly propel it toward malignancy, making these mutations the target of treatment attempts. However, identifying driver mutations can be very challenging due to a variety of complicating factors, including: the presence of many passenger mutations that require distinction from the driver mutation(s); the fact that the collective effects of a group of passenger mutations can alter cancer progression, therefore mimicking a driver mutation; a lack of driver mutations in the section of the tumor that is being sequenced; the presence of both driver and passenger mutations within the same gene; and the fact that driver and passenger mutations may even switch roles (Zhang et al., 2014). In this last regard, it is possible that a driver mutation may be attacked by a targeted cancer treatment at some point, allowing a passenger mutation that is resistant to the treatment to take over (Zhang et al., 2014). All of these factors can add complexity to the hunt for a tumor's driver mutations.

Tumor Profiling Landscape Today

Tumor profiling is treatment-oriented, meaning that identifying driver mutations to inform personalized care is the primary goal. There are several panels currently available that sequence 200-500 cancer-related genes and detect base substitutions, short indels, CNVs, and selected fusions (Frampton et al., 2013). In general, a mutation must be present in greater than 10% of the tumor sample to be detected (Miller, 2012). Most of these panels, including Foundation Medicine's FoundationOne cancer panel – the data from which are used in this study – eliminate germline mutations to narrow the focus of the test (Fang et al., 2014; FoundationOne, 2014). Foundation Medicine conducts this test by interrogating the coding regions of 315 cancer-related genes and select introns from an additional 28 genes for alterations (FoundationOne, 2014). In contrast with many research-based studies,

FoundationOne does not require matched control samples from the patient to filter out germline information and instead removes any germline variants described in the 1000 Genomes website (FoundationOne, 2014; Sun et al., 2014). While company studies have shown the accuracy rate of the method to be above 95% (Sun et al., 2014), there is the potential for germline results to remain in final profiling reports.

The FoundationOne team does not change its interpretation of tumor profiling results based on cancer type (FoundationOne, 2014). This panel and others like it test for genes associated with many different cancers, as tumors have been demonstrated to contain mutations associated with unrelated forms of cancer (Chmielecki et al., 2014; Lipson et al., 2012). Although these mutations may not be the initial cause of the cancer, they may be powerful yet treatable driver mutations brought about as a consequence of cellular instability. For example, in the sequencing of one individual's ovarian tumor, four treatable mutations not commonly associated with ovarian cancer were found using the FoundationOne cancer panel (Lipson et al., 2012). There has been a lot of success with this customized treatment approach, but it is still experimental. Drugs that successfully treat one type of cancer may not be able to do so with another type of cancer. For instance, the therapy that successfully treated *BRAF* driver mutations in patients with melanoma unfortunately did not have the same success treating *BRAF* driver mutations in patients with CRC (Tripathy, Harnden, Blackwell, & Robson, 2014). It is therefore important to consider novel treatment possibilities and opportunities.

Germline Mutations Detected in Tumor Profiling Studies

Approximately 30% of all cancers are caused by an unknown inherited component coupled with environmental carcinogens (Esteban-Jurado et al., 2014). Recent studies have

confirmed that genomic profiling of tumors is able to detect germline mutations and does so regularly. Schrader et al. (2015) of Memorial Sloan Kettering Cancer Center conducted a study in which 1,570 tumors of 68 different types (20% breast cancer; 14% non-small cell lung cancer; 66% other) were sequenced using a 341-gene cancer panel in a research setting; patients provided a matched blood sample as the germline control. Of the germline variants found, 16% were likely pathogenic. Approximately 101 (6.4%) of the 1,570 patients had at least one pathogenic germline mutation, and 807 had at least one VUS. In addition, approximately 13 (0.8%) patients displayed pathogenic germline variants not associated with their cancer type. For example, multiple patients with gastric cancer had pathogenic *BRCA1* mutations, and *BRCA1* is not significantly associated with gastric cancer. These authors believe that germline mutations and variants may play a greater role in tumor development than currently recognized. It also demonstrates the availability of genetic counseling opportunities for 13 patients (with germline pathogenic variants) who most likely would not have been offered counseling or germline testing outside of the research setting. Because the germline mutations were found, these patients now have the option to pursue targeted screening, inform family members of associated risks, and take additional risk-lowering measures. For instance, a patient at increased risk of developing CRC may be recommended to take daily aspirin to combat this risk (Wang et al., 2015). Had this study been done in the clinical setting, it is possible that, based on current lab protocols, these germline variants would have been removed from the data or reported with minimal information such that potentially helpful management changes and disclosure guidelines may not have been shared with the patient.

Another study conducted by Meric-Bernstam and colleagues (2016) of the University of Texas MD Anderson Cancer Center revealed similar results. In this study, 1,000 tumors of various types were sequenced using a 202-gene cancer panel in a research setting, and patients provided a matched control in the form of a blood sample. Meric-Bernstam et al. (2016) reported that 43 (~4.3%) out of the 1,000 patients had at least one pathogenic germline variant in 19 actionable cancer genes, with 16 (~1.6%) of these patients not meeting requirements for germline testing based on clinical information and family history alone. These numbers are relatively consistent with those of Schrader et al. (2015), further demonstrating that patients with cancer tend to have cancer-predisposing germline mutations at a much higher rate than the general population, whether or not those mutations are related to their type of cancer. Again, if these 16 patients were not tested in the research setting, it is likely, based on current recommendations, that they might never have been tested.

Need for Further Research

Few studies have examined the frequency of germline findings in research-based genomic profiling of multiple tumor types (Hall et al., 2015; Meric-Bernstam et al., 2016; Schrader et al., 2015). Building on these data, more research in the clinical context is needed, especially regarding the percentage of patients with germline findings and the percentage that are informed of these results and receive appropriate follow-up.

In addition, we are aware of no studies in the literature that assess the effectiveness of using public databases to gather information on reported tumor sequencing variants.

Although the onus for comprehensive reporting is technically placed on the lab (Dumur, 2014), identification of patients genetically predisposed to develop cancer is in practice done by clinicians, if at all. With over 900 different germline mutations known in the APC gene

alone (Esplin & Snyder, 2014), it may not be feasible for a clinician, such as a genetic counselor, to recognize these variants without referencing databases. Although a plethora of variant databases exists, they are often kept in silos, making it challenging for a clinician to cross-reference multiple databases at once (Rehm et al., 2015). Not only that, but some commercial labs may keep private databases that are not accessible to the public or most genetic counselors. This, coupled with patient demand for testing and workload of a busy clinician, may not allow for extensive germline variant research if not initially reported by the lab.

Methods

Study Design

Through retrospective chart review, we compiled descriptive data of tumor profiling findings in a variety of tumor types by examining FoundationOne results reports. (A matched germline sample is not required in this profiling pipeline.) All reported variants were assessed for evidence of pathogenicity and for the potential of germline origin. Results of the descriptive analyses were used to devise indications for further review of patients' personal and family histories and to identify cases appropriate for a referral for genetic counseling.

Sample Population

Our sample consists of de-identified FoundationOne tumor profiling reports from cancer patients treated at the University of Texas Southwestern Medical Center (UTSMC), Parkland Memorial Hospital and the Children's Medical Center of Dallas. Our sample is representative of a diverse patient population with a variety of tumor types in which tumor profiling was ordered with the purpose of identifying targeted cancer treatments. For the purpose of this project, non-solid tumor samples such as leukemia, bone marrow samples,

and samples which were listed as “unknown primary cancer” reports were not analyzed.

Procedures

Of the 405 total tumor profiling reports obtained, the 22 that were classified by FoundationOne as “failed” were discarded. In addition, leukemia, bone marrow disorder, and unknown primary cancer reports were also discarded, as we chose to examine solid tumors only. This resulted in a final sample size of 160 reports encompassing 66 different tumor types.

Reports were de-identified and assigned a sample ID. For each report, the following information was recorded: sample ID, tumor type, genes in which variants were found, specific variants found, and VUS or mutation status as classified by FoundationOne. We also noted the tumor types in our sample that are present in the ACMG and National Society of Genetic Counselors (NSGC)’s 2014 practice guideline for cancer predisposition referrals (Hampel et al., 2014).

All variants were cross referenced in the Clinvar public database, and the following information was recorded if an entry was found: ClinVar ID, clinical significance rating, date of search, total number of submissions, associated conditions, and number of germline submissions.

A list of selected genes associated with cancer predisposition was curated from existing genetic testing panels available at major commercial genetic testing companies (Table 1 in Appendix). Genes associated with Fanconi Anemia, a recessive cancer-predisposing syndrome, were also included, bringing the total to 86 genes. All variants in our sample found in genes present in this curated list were noted for analysis, as they have each been associated with cancer predisposition.

Measures and Data Analysis

Criteria for referrals to genetic counseling required either a tumor type from the ACMG/NSGC's list of referral indications for cancer predisposition assessment (Hampel et al., 2014) or a mutation that has been previously observed in the germline as pathogenic, as noted in the ClinVar database, in a gene associated with cancer predisposition (Table 1). All variants labeled as "mutation" in FoundationOne reports were presumed to be pathogenic. All variants labeled as "variant of unknown significance" in FoundationOne reports were deemed suspicious for germline pathogenicity if they were described as "pathogenic," "likely pathogenic," or "risk factor" in ClinVar, regardless of other conflicting calls, and ClinVar reserves the "risk factor" designation "for variants that are interpreted not to cause a disorder but to increase the risk" ("Relating variation to medicine," n.d.). All data were compiled in Microsoft Excel.

Results

Our sample of 160 tumor genomic profiling reports comprised 26 breast invasive ductal carcinoma (IDC) reports, 16 breast carcinoma (NOS) reports, 10 brain glioblastoma (GBM) reports, and 108 reports of 63 other tumor types, made up of 5 or fewer reports each. Mutations and VUSs were described by tumor type (Table 2 in Appendix), and reports averaged 4.24 mutations (0 - 13) and 9.8 VUSs (0 - 44) per individual tumor.

We examined reports for genetic counseling referral indications based on: (1) current ACMG/NSGC guidelines for tumor type, (2) mutations reported, and (3) VUSs reported. We then compared methods of detection.

After evaluating all 160 reports based on ACMG/NSGC guidelines for tumor type, it was determined that: 9 (6%) met criteria for a referral to genetic counseling based solely on

tumor type; 116 (72%) warranted assessment of personal and familial risk factors to determine whether a genetic counseling referral was appropriate based on tumor type; and 35 (22%) had no indication for a referral to genetic counseling or further assessment based on tumor type (Hampel et al., 2014).

We examined our sample based on individual mutations and VUSs reported and described the utility of searching for variants in public database ClinVar (Table 3 in Appendix). Of the 679 individual mutations reported across our sample, 274 (40%) occurred in genes associated with cancer predisposition syndromes. Though we were unable to find 222 (81%) of these 274 mutations in the ClinVar database, we were able to find 52 (19%) – with 48 (18%) reported as germline at least once and 42 (15%) reported as both germline and either “pathogenic,” “likely pathogenic,” or “risk factor” at least once – thereby identifying a group of mutations which, if present in the germline, would be concerning for a hereditary cancer predisposition syndrome.

We evaluated this group of at-risk mutations in the context of current ACMG/NSGC guidelines, in order to determine if current tumor type screening identified them. The 42 mutations that were each reported at least once as both germline and on the pathogenic spectrum originated from 39 separate reports. Of these 39 reports: none met criteria for a referral to genetic counseling based solely on tumor type; 34 warranted assessment of personal and familial risk factors to determine whether a genetic counseling referral was appropriate based on tumor type; and 8 had no indication for a referral to genetic counseling or further assessment based on tumor type (Hampel et al., 2014). The latter 8 reports were described in further detail (Table 4 in Appendix).

As the majority of mutations in cancer predisposition genes could not be cross-referenced in ClinVar, we looked at the total sample of reports containing mutations in cancer predisposition genes for comparison of gene-focused methods (of screening for genetic counseling referral indications) with tumor type-focused methods. Of our 160 total reports, 127 reports (79%) carried mutations in genes associated with cancer predisposition. Of these reports, 5 (4%) met criteria for a referral to genetic counseling based solely on tumor type [adrenal gland cortical carcinoma (2), ovary sex-cord stromal tumor (1), pediatric soft tissue rhabdomyosarcoma alveolar (1), and soft tissue paraganglioma (1)]; 93 (73%) warranted assessment of personal and familial risk factors to determine whether a genetic counseling referral was appropriate based on tumor type; and 29 (23%) had no indication for a referral to genetic counseling or further assessment based on tumor type alone (Hampel et al., 2014).

We found that 104 reports (65%) had mutations in genes associated with cancer predisposition syndromes. When *TP53* – a gene with mutations that are often somatic in origin – was excluded, the 79% of reports that contained mutations in genes associated with cancer predisposition decreased by 14% to the 65% that we now report (Olivier et al., 2010).

Of the 1,569 VUSs reported across our sample, 360 (23%) occurred in genes associated with cancer predisposition. Though we were unable to find 248 (69%) of these 360 VUSs in ClinVar, we were able to find 112 (31%) – with 7 (2%) reported at least once as “pathogenic,” “likely pathogenic,” or “risk factor” [*APC*, *BLM*, *BRIP1*, *CHEK2*, *PTEN*, *RET*, and *TSCI*]. All 7 of these VUSs also had at least one germline entry in ClinVar.

We examined this group of VUSs in the context of ACMG/NSGC guidelines for tumor type. All 7 of these reports met criteria for assessment of personal and familial risk

factors to determine whether a genetic counseling referral was appropriate based on tumor type alone (Hampel et al., 2014). Further details of the reports were described (Table 5 in Appendix).

In summary, of the combined total of 2,248 mutations and VUSs reported in our sample, 55 (over 2%): were labeled in ClinVar at least once as “pathogenic,” “likely pathogenic,” or “risk factor;” had at least one germline entry in ClinVar; and occurred in a gene associated with cancer predisposition.

Discussion

Results in the Context of Current Recommendations

The ACMG and NSGC currently recommend referral of individuals with certain tumor types (such as ovary) for genetic counseling – regardless of age, family history, and other factors – as well as further examination of personal and family medical history for other cancer types (such as breast) (Hampel et al., 2014). Given our findings, our study’s method of assessing cancer predisposition syndrome risk based on gene rather than on tumor type identified an additional group of patients with mutations that warrant evaluation for a cancer predisposition syndrome. This suggests that tumor profiling has the potential to identify high-risk patients that remain undetected by current screening recommendations for tumor type alone. It is therefore our opinion that screening for genetic counseling referral indications be both tumor type-focused and gene-focused when applicable.

Results in the Context of Similar Studies

As the present study did not include a matched germline control, we have no way of knowing which genetic alterations detected in our sample, if any, are germline in origin. However, previous studies can provide insight on the percentage of tumors in our sample that

might possess germline mutations in cancer predisposition genes. National statistics report that 5-10% of breast cancers and 5-10% of cancers in general are caused by a germline mutation (“Genetics of Breast and Gynecologic Cancers,” n.d.; “The Genetics of Cancer,” n.d.), while tumor profiling studies that included a matched germline control have found germline mutations in cancer predisposition genes in 3-6% of reports (Jones et al., 2015; Meric-Bernstam et al., 2016; Schrader et al., 2015). These findings indicate that some germline mutations are detected through tumor profiling and that there is a need for tumor profiling reports generated without utilization of a matched germline control to be assessed for genetic counseling referral indications.

In the current study, we found that the majority of mutations in genes associated with cancer predisposition syndromes that were found in ClinVar had at least one germline entry. This suggests that the tumor profiling methods used in our study, much like previous studies, detected both germline and somatic findings.

Our study was unlike previous studies in that we did not have access to matched germline controls for our samples. We therefore had to do our best in estimating which mutations carried the highest risk of being associated with germline cancer predisposition. In the process, we discovered that tumor type-focused and gene-focused screening methods identify different patients as at-risk, with some overlap. As previously stated, we therefore believe that tumor profiling reports generated without utilization of a matched germline control should be reviewed based on individual mutations and variants found, in addition to tumor type. Genetic counseling can help to further clarify which patients may truly be at risk for a germline pathogenic disease through risk evaluation and subsequent germline testing.

Implications for Providers

Results of this study describe the clinical utility of searching public database ClinVar for mutations and VUSs cited in tumor profiling reports. Of alterations occurring in cancer predisposition genes, there was a 31% likelihood of finding a VUS and 19% likelihood of finding a mutation entered in ClinVar, and there was a 2% likelihood of finding a VUS and 17% likelihood of finding a mutation with at least one entry on the pathogenic spectrum in ClinVar. These findings confirm that ClinVar is primarily a database of yet-to-be-categorized variants and that its calls may be inconsistent with those of FoundationOne.

The process of researching alterations occurring in cancer predisposition genes in ClinVar identified 8 patients in our study with mutations – which, if present in the germline, would be concerning for hereditary cancer predisposition syndromes – who had no indication for a referral to genetic counseling or further assessment based on tumor type. An additional 7 patients with similarly concerning VUSs who would not have received referrals to genetic counseling based on tumor type alone were also identified. This suggests that there is value in researching both mutations and VUSs reported in tumor genomic profiling.

Depending on a provider's caseload, familiarity with searching for genetic variants, knowledge of a patient's personal and familial history, knowledge of this study, and other factors, she or he may decide differently on whether additional researching of variants in public databases is appropriate. This inconsistency in care decision-making may potentially diminish if tumor profiling companies change their reporting structure to include either: more information on variants found in genes associated with cancer predisposition or a matched germline control.

Another aspect of the study that is particularly relevant to providers is the fact that we had very limited knowledge about the patients whose tumor reports we reviewed, which may often be realistic in the clinical setting. One can imagine many situations in which having access to a patient's detailed family and personal history may be difficult. For example, a patient may have limited knowledge of her or his family history or may have recently transferred care without previous medical documents available. In situations where access to a patient's age, sex, and other personal factors is available, this information may quickly be used to rule in or out genetic counseling referrals. Other factors that are less likely to be known – such as ethnicity, cancer history, or family history – may also be used in quick assessments of genetic risk. It is in the absence of all such knowledge that the methods described in this study may prove most useful.

Study Limitations

The primary limitation of our study is that we are unable to confirm suspected germline mutations against a matched control. This is the inherent bias of tumor profiling conducted without matched control and the reason why the true percentage of germline mutations and variants is unknown in this study.

Another limitation was our usage of a single database – ClinVar – in gathering information on pathogenicity and germline origin. We did not use other methods such as functional assays, in silico predictors, or other variant databases. We acknowledge that there may be variants that are more accurately assessed in other gene-specific databases, and there may be stricter regulation of entries in non-public databases. ClinVar was primarily utilized in this study due to its status as a large public resource that is readily available to a variety of professionals who may be interpreting tumor profiling reports. Therefore, we anticipated that

our methods would have the potential to be widely replicated by professionals that wish to do so.

In addition, variants with only 1 pathogenic entry in ClinVar were categorized the same as variants with 10 pathogenic entries in ClinVar, as no feasible weight scale could be applied in analyses. This limitation allows variants that have been observed as pathogenic only once to be assigned to a risk category, which is a broad method of categorizing variants. This method, however, may be appropriate for clinicians looking to determine if genetic counseling is warranted based on tumor profiling results alone.

With regards to our list of genes associated with cancer predisposition, its large size exceeds the ACMG's recommended list for reporting of incidental findings (Green et al. 2013), leaving some genes without clear recommendations for medical management. Despite this, we felt that the curated list was representative of today's market of germline testing products. As multi-gene cancer panels become more commonplace and our understanding of these genes expands, patients may opt for this more comprehensive method of testing following genetic counseling. This shift may result in more conversations and discoveries around panel gene findings.

Conclusions

Tumor profiling conducted without a matched germline control has the potential to detect germline mutations and therefore identify patients at increased risk for hereditary cancer predisposition syndromes. By assessing FoundationOne tumor profiling reports for mutations in genes associated with these syndromes, we identified patients who warrant further risk evaluation than current guidelines – based on tumor type alone – indicate. This suggests that tumor profiling reports generated without a matched germline control should be

reviewed based on individual mutations and VUSs found, in addition to tumor type. We also found – for some of the reported mutations and VUSs – supplemental information on pathogenicity and germline origin in public database ClinVar. This suggests that there is value in cross-referencing tumor profiling alterations in ClinVar, though calls were not always consistent with those of FoundationOne.

Appendix**Table 1: Selected genes list (compilation of genes associated with hereditary cancer predisposition)**

<i>ALK</i>	<i>EGFR</i>	<i>NF2</i>	<i>SDHAF2</i>
<i>APC</i>	<i>ENG</i>	<i>PALB2</i>	<i>SDHB</i>
<i>ATM</i>	<i>EPCAM</i>	<i>PAX5</i>	<i>SDHC</i>
<i>AXIN2</i>	<i>FH</i>	<i>PDGFRA</i>	<i>SDHD</i>
<i>BAP1</i>	<i>FLCN</i>	<i>PHOX2B</i>	<i>SMAD4</i>
<i>BARD1</i>	<i>GATA2</i>	<i>PIK3CA</i>	<i>SMARCA4</i>
<i>BLM</i>	<i>GPC3</i>	<i>PMS2</i>	<i>SMARCB1</i>
<i>BMPR1A</i>	<i>GREM1</i>	<i>POLD1</i>	<i>SMARCE1</i>
<i>BRCA1</i>	<i>HOXB13</i>	<i>POLE</i>	<i>STK11</i>
<i>BRCA2</i>	<i>HRAS</i>	<i>PRF1</i>	<i>SUFU</i>
<i>BRIP1</i>	<i>KIT</i>	<i>PRKARIA</i>	<i>TERC</i>
<i>CASR</i>	<i>MAX</i>	<i>PTCH1</i>	<i>TERT</i>
<i>CDC73</i>	<i>MEN1</i>	<i>PTEN</i>	<i>TMEM127</i>
<i>CDH1</i>	<i>MET</i>	<i>PTPN11</i>	<i>TP53</i>
<i>CDK4</i>	<i>MITF</i>	<i>RAD50</i>	<i>TSC1</i>
<i>CDKN1B</i>	<i>MLH1</i>	<i>RAD51C</i>	<i>TSC2</i>
<i>CDKN1C</i>	<i>MRE11A</i>	<i>RAD51D</i>	<i>TSHR</i>
<i>CDKN2A</i>	<i>MSH2</i>	<i>RB1</i>	<i>VHL</i>
<i>CEBPA</i>	<i>MSH6</i>	<i>RECQL4</i>	<i>WRN</i>
<i>CHEK2</i>	<i>MUTYH</i>	<i>RET</i>	<i>WT1</i>
<i>DICER1</i>	<i>NBN</i>	<i>RUNX1</i>	
<i>DIS3L2</i>	<i>NF1</i>	<i>SDHA</i>	

Table 2: Mutation and VUS frequency by tumor type

	Tumor type	# of patients	Total # of mutations	Total # of VUSs	Avg. # of mutations per patient	Avg. # of VUSs per patient	Of genes affected multiple times within tumor type group, gene most commonly affected (# of times affected)
1	adrenal gland cortical carcinoma	2	3	14	1.5	7	<i>MLL2</i> (2)
2	adrenal gland neuroblastoma	3	5	13	1.7	4.3	<i>ARID1A</i> (2), <i>NOTCH3</i> (2)
3	appendix adenocarcinoma	1	4	12	4	12	None
4	bladder adenocarcinoma	1	2	2	2	2	None
5	bladder urothelial (transitional cell) carcinoma	1	7	0	7	0	None
6	bone chordoma	1	1	3	1	3	None
7	bone osteosarcoma	1	2	8	2	8	<i>SMARCB1</i> (2)
8	brain anaplastic astrocytoma	1	6	10	6	10	None
9	brain astrocytoma pilocytic	1	1	4	1	4	None
10	brain astrocytoma pilomyxoid	1	1	7	1	7	None
11	brain ependymoma	1	2	4	2	4	<i>MSH3</i> (2)
12	brain glioblastoma (GBM)	10	46	65	4.6	6.5	<i>EGFR</i> (7)
13	brain glioma (NOS)	2	11	8	5.5	4	<i>FGFR1</i> (2)
14	brain medulloblastoma	1	3	11	3	11	<i>LRP1B</i> (2), <i>SPEN</i> (2)

15	breast carcinoma (NOS)	16	101	215	6.3	13.4	<i>PIK3CA</i> (9)
16	breast invasive ductal carcinoma (IDC)	26	306	534	11.8	20.5	<i>TP53</i> (32)
17	breast invasive lobular carcinoma (ILC)	2	10	12	5	6	<i>CDHI</i> (2), <i>MED12</i> (2), <i>PIK3CA</i> (2)
18	colon adenocarcinoma (CRC)	2	20	57	10	28.5	<i>PRKCI</i> (4)
19	esophagus adenocarcinoma	3	16	50	5.3	16.7	<i>LRP1B</i> (3)
20	eye intraocular melanoma	1	6	4	6	4	<i>BAP1</i> (2)
21	head and neck squamous cell carcinoma (HNSCC)	1	4	8	4	8	None
22	kidney clear cell carcinoma	1	4	11	4	11	<i>TET2</i> (2)
23	kidney urothelial carcinoma	1	1	7	1	7	<i>ERBB2</i> (2)
24	liver cholangiocarcinoma	2	6	10	3	5	None
25	liver hepatoblastoma	1	2	27	2	27	<i>ASXL1</i> (2)
26	liver hepatocellular carcinoma (HCC)	1	2	22	2	22	None
27	lung adenocarcinoma	4	18	31	4.5	7.7	<i>BRCA2</i> (2), <i>DDR2</i> (2), <i>KRAS</i> (2), <i>MRE11A</i> (2), <i>NF1</i> (2), <i>POLE</i> (2), <i>RICTOR</i> (2), <i>SPTA</i> (2), <i>STK11</i> (2)
28	lung large cell neuroendocrine carcinoma	1	4	12	4	12	None
29	lung small cell undifferentiated carcinoma	3	8	61	2.7	20.3	<i>TP53</i> (3)

30	lung squamous cell carcinoma (SCC)	1	4	11	4	11	None
31	myeloid sarcoma	1	1	10	1	10	None
32	nasopharynx and paranasal sinuses esthesioneuroblastoma	1	0	6	0	6	None
33	ovary serous carcinoma	3	6	25	2	8.3	<i>KRAS</i> (2)
34	ovary sex-cord stromal tumor	1	2	7	2	7	<i>DICER1</i> (2)
35	pancreas ductal adenocarcinoma	5	19	35	3.8	7	<i>KRAS</i> (5), <i>TP53</i> (5)
36	pancreas solid and papillary tumor	1	3	2	3	2	None
37	pancreatobiliary carcinoma (NOS)	1	3	7	3	7	None
38	pediatric adrenal gland neuroblastoma	1	6	10	6	10	<i>ALK</i> (5)
39	pediatric brain astrocytoma	1	6	15	6	15	<i>BRD4</i> (2)
40	pediatric brain astrocytoma pilomyxoid	1	1	6	1	6	None
41	pediatric liver hepatoblastoma	1	2	11	2	11	None
42	pediatric soft tissue rhabdomyosarcoma alveolar	1	2	11	2	11	<i>STAT6</i> (2)
43	pediatric soft tissue sarcoma (NOS)	1	1	7	1	7	<i>WDR90</i> (2)
44	penis squamous cell carcinoma (SCC)	1	4	18	4	18	<i>RANBP2</i> (2), <i>RET</i> (2)
45	rectum adenocarcinoma (CRC)	1	3	3	3	3	None
46	salivary gland acinic cell tumor	1	1	0	1	0	None

47	skin adnexal carcinoma	1	6	11	6	11	None
48	skin basal cell carcinoma	1	5	22	5	22	<i>LRP1B</i> (3)
49	skin melanoma	2	10	50	5	25	<i>ALK</i> (3), <i>PRKDC</i> (3)
50	skin squamous cell carcinoma (SCC)	2	13	17	6.5	8.5	<i>TP53</i> (6)
51	soft tissue angiosarcoma	1	0	11	0	11	None
52	soft tissue chondrosarcoma	3	9	36	3	12	<i>BRCA2</i> (2), <i>MLL3</i> (2), <i>PRKARIA</i> (2)
53	soft tissue ewing sarcoma	1	2	7	2	7	None
54	soft tissue leiomyosarcoma	4	11	26	2.7	6.5	<i>TP53</i> (3)
55	soft tissue liposarcoma	1	4	13	4	13	None
56	soft tissue malignant peripheral nerve sheath tumor (MPNST)	3	13	34	4.3	11.3	<i>CDKN2A</i> (2), <i>CUX1</i> (2), <i>KDM4C</i> (2), <i>SUZ12</i> (2), <i>WDR90</i> (2)
57	soft tissue neuroblastoma	4	4	21	1	5.2	<i>ATM</i> (2), <i>PIK3CG</i> (2)
58	soft tissue paraganglioma	1	2	3	2	3	<i>ATRX</i> (2)
59	soft tissue rhabdomyosarcoma (NOS)	2	9	24	4.5	12	<i>EP300</i> (2), <i>FANCD2</i> (2), <i>MKI67</i> (2)
60	soft tissue sarcoma (NOS)	4	7	33	1.7	8.2	<i>GPR124</i> (2)
61	soft tissue schwannoma	1	2	6	2	6	None
62	stomach adenocarcinoma diffuse type	1	3	7	3	7	<i>CDHI</i> (2)

63	thyroid anaplastic carcinoma	5	31	46	6.2	9.2	<i>TP53</i> (11)
64	unknown primary melanoma	5	27	41	5.4	8.2	<i>BAP1</i> (2), <i>BRAF</i> (2), <i>FAM123B</i> (2), <i>FGF3</i> (2), <i>HGF</i> (2), <i>KDR</i> (2), <i>MAP2K1</i> (2), <i>MCL1</i> (2), <i>MYC</i> (2), <i>RUNX1T1</i> (2)
65	unknown primary urothelial carcinoma	1	5	9	5	9	None
66	urethra squamous cell carcinoma (SCC)	1	3	13	3	13	<i>CDKN2A</i> (2), <i>MLL2</i> (2)

Table 3: ClinVar reporting of FoundationOne mutations and VUSs

	Total #	Total # (%) in selected genes	Total # (%) of those in selected genes that were found in ClinVar	Total # (%) of those in selected genes that were cited ≥ 1 times as <i>pathogenic, likely pathogenic, or risk factor</i> in ClinVar	Total # (%) of those in selected genes that were cited as <i>benign or likely benign</i> only in ClinVar
Mutations	679	274 (40.4)	52 (19)	46 (17)	0 (0)
VUSs	1,569	360 (23)	112 (31)	7 (2)	21 (5.8)

Table 4: Selected reports with mutations in cancer predisposition genes and no indications for referrals to genetic counseling or further assessment based on tumor type alone

	Tumor type	Gene containing mutation	Change detected	Total # of submissions in ClinVar	Total # of germline submissions in ClinVar	ClinVar clinical significance
1	bladder urothelial (transitional cell) carcinoma	<i>PIK3CA</i>	c.1624G>A	4	1	<i>Pathogenic</i>
2	liver cholangiocarcinoma	<i>APC</i>	c.3920T>A	8	8	<i>Conflicting interpretations of pathogenicity, not provided, risk factor; Pathogenic (2); Uncertain significance (1)</i>
3	lung adenocarcinoma	<i>BRCA2</i>	c.476-1G>A	2	2	<i>Pathogenic</i>
4	penis squamous cell carcinoma (SCC)	<i>RET</i>	c.2410G>A	4	4	<i>Pathogenic</i>
5	skin squamous cell carcinoma (SCC)	<i>TP53</i>	c.844C>T	5	5	<i>Conflicting interpretations of pathogenicity; Likely benign (2); Pathogenic (3)</i>
6	skin squamous cell carcinoma (SCC)	<i>TP53</i>	c.844C>T	5	5	<i>Conflicting interpretations of pathogenicity; Likely benign (2); Pathogenic (3)</i>
7	soft tissue neuroblastoma	<i>PTPN11</i>	c.1508G>C	1	1	<i>Pathogenic</i>
8	soft tissue neuroblastoma	<i>ALK</i>	c.3824G>A	3	2	<i>Pathogenic, risk factor</i>

Table 5: Selected reports with VUSs in cancer predisposition genes and tumor types meeting criteria for assessment of personal and familial risk factors

	Tumor type	Gene containing VUS	Change detected	Total # of submissions in ClinVar	Total # of germline submissions in ClinVar	ClinVar clinical significance
1	breast invasive ductal carcinoma (IDC)	<i>APC</i>	c.388A>G	4	4	<i>Conflicting interpretations of pathogenicity; Likely pathogenic (1); Pathogenic (4); Uncertain significance (1)</i>
2	breast invasive ductal carcinoma (IDC)	<i>BLM</i>	c.2015A>G	1	1	<i>Likely pathogenic</i>
3	breast invasive ductal carcinoma (IDC)	<i>BRIP1</i>	c.1045G>C	3	3	<i>Conflicting interpretations of pathogenicity, not provided; Pathogenic (1); Uncertain significance (1)</i>
4	breast invasive ductal carcinoma (IDC)	<i>CHEK2</i>	c.1283C>T	4	4	<i>Pathogenic, risk factor</i>
5	lung squamous cell carcinoma (SCC)	<i>PTEN</i>	c.70G>C	1	1	<i>Pathogenic</i>
6	unknown primary melanoma	<i>RET</i>	c.2372A>T	14	14	<i>Conflicting interpretations of pathogenicity; Benign (2); Likely benign (2); Pathogenic (8); Uncertain significance (2)</i>
7	unknown primary melanoma	<i>TSC1</i>	c.2194C>T	9	9	<i>Conflicting interpretations of pathogenicity, not provided; Benign (3); Likely benign (2); Pathogenic (1); Uncertain significance (1)</i>

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