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REVIEW ARTICLE

Somatic mutation panels: Time to clear their names

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Abstract

With improvements in DNA sequencing technologies and the consequent reduction in costs, next generation sequencing is being utilized increasingly in panel-based testing to perform molecular profiling of tumors. Such tumor-based panels are often referred to as 'somatic' panels, but this term is misleading and should not be used, since not all DNA variants within a tumor are somatic in nature. Every cell in a person's body contains that person's germline DNA, including tumor cells. Moreover, tumor samples are invariably contaminated with blood, a tissue that can contain somatic mutations itself in a process now called clonal hematopoiesis. Differentiating between germline variants or tumor-associated somatic mutations versus clonal hematopoiesis can be challenging. In this review, we address how to interpret the results of somatic mutation panels, how to differentiate between germline and truly somatic events, and discuss the importance of this distinction.

Keywords Germline predisposition, Next generation sequencing, Cancer, Somatic variant, Germline variant, Clonal hematopoiesis.

A representative case

A 65 year old woman with a history of an ER+ PR+ HER2+ invasive ductal carcinoma of the right breast diagnosed at age 34, status-post lumpectomy/radiation/and chemotherapy, presented to the Hematology Clinic with newly diagnosed chronic lymphocytic leukemia (CLL). Panel-based next generation sequencing (NGS) was conducted on the peripheral blood and revealed a *TP53* (c.743G>A, p.R248Q) mutation with a variant allele fraction (VAF) of 45%. Due to anemia and constitutional symptoms, she began treatment with ibrutinib. She achieved a complete remission but had minimal residual disease (MRD). Six months later, she developed abnormal uterine bleeding. Further investigation uncovered a small Fallopian tube tumor, which was completely excised. An NGS panel performed on the formalin fixed paraffin embedded (FFPE) Fallopian tube tumor showed the same *TP53* (c.743G>A, p.R248Q) mutation, but this time with a VAF of 90%.

The gynecologist calls you to discuss the *TP53* mutation. She wants to know if the patient has Li-Fraumeni Syndrome (LFS) and should be referred to a genetics counselor. She is cautious, because her resident thinks that the variant is coming from contaminating CLL cells, and the medical student who had just finished his Hematology/Oncology rotation challenged them both, saying that this could be due to clonal hematopoiesis (CH), given her age. She asks you what you think.

Germline variants as incidental findings

Tumor-based NGS panels are becoming more commonplace and can help inform diagnosis, prognosis, and treatment decisions, furthering the goal of precision oncology. However, caution is advised in the interpretation of so-called 'somatic' NGS panels, because all cells in a person's body contain

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Table 1 Pathogenic germline variants as incidental findings from tumor-based sequencing.

Reference	Tumor type	Number of genes evaluated	Number of patients	Number of patients with PGVs	Percentage of patients with PGVs
[3]	Solid tumor and hematologic	85	815	27	3%
[4]	Solid tumors and hematologic	36	439	19	4.3%
[5]	Solid tumors and lymphoma	19	1000	43	4.3%
[6]	Gastroesophageal, hepatobiliary, and colorectal	NR (NGS FoundationOne panel testing)	7	3	43%
[7]	Solid tumors	16	10,888	156	1.4%
[8]	Advanced solid tumors	187	1566	246	15.7%
[9]	Advanced solid tumors	76	1040	205	19.7%
[10]	Solid tumors and hematologic	152	10,389	853	8%
[11]	Hematologic	9	52 PVs	6 PGVs	12%*

NGS, next generation sequencing; NR, not reported; PGV, pathogenic or likely pathogenic germline variant; PV, pathogenic variant. *Percentage of total identified pathogenic variants that were found to be germline.

germline DNA, including tumors. As a result, these panels not only detect tumor-specific, acquired variants, but may also incidentally identify germline variants and cannot distinguish between the two, a fact that is important for clinicians ordering and disclosing results of these panels to recognize [1,2].

Frequency of incidental germline findings

Recently, several studies have investigated the frequency of incidental germline variants found during tumor-based sequencing (Table 1) [3–11]. Jones et al. conducted whole exome sequencing (WES) or targeted NGS of 815 patients with apparently sporadic cancers, spanning 15 different types of tumors, including solid tumors and hematologic malignancies [3]. By performing sequencing on paired tumor-normal samples from each of these patients, they were able to identify 3% (27 patients) harboring a germline truncating alteration in at least one of 85 evaluated genes known to be associated with cancer predisposition syndromes. Six mutations in *BRCA1* accounted for 22% of the identified germline variants, and patients with breast and/or ovarian cancer were the most common carriers of these *BRCA1* variants (5 of the 6 cases). A major limitation of this study was that only truncating alterations, including nonsense alterations, splice site changes, and insertions or deletions causing a frameshift were reported. Missense mutations, a common type of germline alteration, were not evaluated and consequently, the reported 3% of patients found to carry a germline mutation in a cancer predisposing gene is likely an underestimate. Despite its limitations, this was one of the first large studies that highlighted the importance of using germline tissue to identify and interpret variants found from tumor-based testing accurately.

Similar to the Jones paper, Seifert et al. found 4.3% of cancer patients had likely pathogenic or known pathogenic germline variants (PGVs) present in somatic sequencing panels conducted on 439 unselected cancer patients with 10 different cancer types, including solid tumors and hematologic malignancies [4]. *BRCA1/2* followed by *ATM* were the most commonly mutated, with 11/19 (58%) and 4/19 (21%) respectively, of the PGVs identified. The majority of the PGVs (12/19) found in this study occurred in genes for which alterations are known to be associated with the cancer type of the patient being studied, with *BRCA1/2* PGVs in patients with breast cancer being the most common. In a slightly larger analysis of 1000 patients with metastatic or locally advanced solid tumors or lymphomas, Meric-Bernstam et al. used paired tumor-normal (blood) tissues to evaluate incidental PGVs in 19 potentially actionable cancer-associated genes from a somatic genomic profiling protocol [5]. Like Seifert, Meric-Bernstam et al. found that 4.3% of patients had a likely PGV, with PGVs in *BRCA1, BRCA2,* and *TP53* being the most prevalent, accounting for 26%, 23%, and 23% of the PGVs, respectively. Ovarian cancer samples were found most commonly to contain PGVs at a rate of 14%. The numbers reported by Seifert and Meric-Bernstam, however, are likely underestimates of the true frequency, because variants were only checked in 36 and 19 hereditary cancer susceptibility genes, respectively.

The reported frequency of PGVs as a secondary finding from tumor-based sequencing varies significantly, from a low of 1.4% to a high of 43% [6,7]. Logically, studies that evaluated a larger number of genes and those that preselected patients based on suspicion for germline predisposition tended to report higher frequencies of PGVs. Examining a broad gene panel, including 187 genes with Mendelian disease associations, Schrader et al., found PGVs in 15.7% of 1566 patients with various advanced solid tumors [8]. When their analysis was restricted to variants within cancer susceptibility

genes and clinically actionable genes, genes for which targeted therapies exist or are in development, the frequency of presumed PGVs decreased to 12.6% and 5.0%, respectively. The most commonly mutated cancer susceptibility genes in this study were *BRCA2* (31 PGVs), *CHEK2* (23 PGVs), *MUTYH* (23 PGVs), and *BRCA1* (21 PGVs). Mandelker et al. performed paired tumor-normal sequencing on 1040 patients with advanced solid tumors and found that 205 (19.7%) carried PGVs within 76 evaluated cancer susceptibility genes [9]. Out of the 205 PGVs, 45 and 28 were within the *BRCA2* and *CHEK2* genes, respectively. They also determined that 101 clinically actionable PGVs would have been missed if guideline-directed germline testing, based on family history, age at diagnosis, and tumor type, were used rather than paired tumor-normal sequencing. In one of the largest investigations into PGVs in cancer to date, Huang et al. used WES filtered for 152 cancer susceptibility genes on paired tumor-normal samples to search for secondary PGVs within 10,389 individuals with a variety of solid tumors and hematologic malignancies [10]. Eight percent of patients were found to carry a PGV, with the highest rates of PGVs in ovarian cancer and pheochromocytoma and paraganglioma tumors at 19.9% and 22.9%, respectively. In addition to the larger number of genes evaluated, the investigators of this study examined not only single nucleotide variants (SNVs) but also copy number variants (CNVs), which could account for the higher frequency of PGVs found compared to earlier studies. Together these studies demonstrate that PGVs are frequent incidental findings in tumor-based sequencing, and the frequency at which PGVs are detected depends on the comprehensiveness of the analysis.

The aforementioned studies all involved solid tissue tumor specimens. Naturally though, PGVs can be found incidentally in other tumor sources, including liquid biopsies. 156 of 10,888 patients (1.4%) with advanced stage solid tumors were found to have a putative PGV on tumor-based NGS using cell free DNA (cfDNA) [7]. In this study, the search for PGVs was limited to 16 actionable hereditary cancer genes, mutations with VAFs less than 40% were excluded, equivocal variants from samples with high circulating tumor DNA load (defined by the authors as more than one circulating tumor mutation present at an allelic frequency greater than 30%) were excluded, and germline CNVs were not evaluated, all of which may account for the low prevalence compared to those found when using DNA from solid tissue. The potential impact of the number of circulating tumor cells on the frequency of PGV detection was not addressed in this article. The highest prevalence of PGVs in this study were found in ovarian, prostate, and pancreatic cancer at 8.1%, 3.5%, and 3.3%, respectively. This study shows that sequencing cfDNA has the same challenges of incidental germline variant detection as does solid tissue tumor-based sequencing, and caution with variant assignment must be taken in both cases.

In addition to somatically mutated genes, NGS panels conducted for hematologic neoplasms also contain genes known to be altered in hereditary hematologic malignancies (HHM) [12,13]. It would therefore be expected that such panels performed on the peripheral blood or bone marrow of patients with hematologic malignancies may detect both acquired variants and PGVs. This was confirmed by Drazer et al. through review of NGS panels conducted on 360 patients with hematologic malignancies [11]. A total of 52 pathogenic or likely pathogenic variants in 9 HHM-associated genes, among 44 patients with available germline tissue for paired analysis, were identified and 6 (12%) of these variants were confirmed to be of germline origin. PGVs in *DDX41, GATA2,* and *TP53* were identified.

How does knowing the germline status of the variant impact management?

Distinction between somatic and germline aberrations is important as proper assignment of a variant has significant impact on the patient's management and has important implications for their family members [1,13–15]. Patients should be properly counselled prior to sending these tests, and their wishes for disclosure of potential germline findings should be ascertained [16].

For example, incidentally finding a pathogenic germline *TP53* variant can alter a patient's treatment plan for their active malignancy, such as bilateral total mastectomy for breast cancer as opposed to lumpectomy and radiation, to decrease risk of secondary malignancies; impact cancer screening recommendations, such as breast cancer screening with MRI starting around age 25 years and enhanced colorectal cancer screening with colonoscopy every 2–5 years; and should prompt investigation and screening of potentially affected family members [17]. In the case of hematologic malignancies, such as acute myeloid leukemia (AML), a condition which often necessitates an allogeneic stem cell transplant for the best chance of cure, the recognition of a predisposing germline alteration is vital to proper stem cell donor selection. The preferred donor for an allogeneic stem cell transplant is usually a matched sibling donor. However, if the patient carries a germline variant associated with an HHM inherited from a parent, there is a 50% chance that each of their siblings also carries this variant, and use of their stem cells, if affected, would result in reconstitution of the patient's bone marrow with the same leukemia predisposition variant, putting the recipient at risk for complications such as delayed or failed engraftment and donor-derived leukemias [15,18]. Moreover, bone marrow stimulation and stem cell mobilization may increase the donor's inherent risk of developing a HM if the related donor shares the familial mutation. As a result, discovery of such a variant should prompt testing of related donors to ensure use of an unaffected donor or to prompt the use of an unrelated donor.

Just as knowing the germline status of a patient has important implications for the patient's clinical management, so too does the knowledge of tumor-derived somatic mutations. For example as we introduced earlier, in CLL, somatic *TP53* mutations are high risk, portending inferior responses to traditional chemoimmunotherapy, and when present, alternative therapies are suggested [19]. Many therapies targeted against specific acquired mutations now exist, such as vemurafenib, which targets a key driver mutation in hairy cell leukemia (*BRAF* V600E) [20]. However, if the provider conveying the results of genetic testing does not realize that just because the panel is called a 'somatic' panel, not all variants called are in fact somatic, they could easily falsely assign a significant proportion of variants. Many ways to help determine the actual source of a variant have been proposed.

Differentiating between somatic and germline variants

Personal and family history

Suspicion for a germline predisposition to cancer should arise when the diagnosis occurs at a significantly younger age than would normally be expected. Importantly however, presentation at a 'normal' age does not preclude presence of a germline predisposition. For example, myeloid neoplasias associated with PGVs in *DDX41* typically occur at ages no different than the population-based median age at diagnosis [21]. Many known germline cancer predisposition genes present within individuals with a wide age range. For example, in those with germline *RUNX1* mutations, the age of onset of hematological malignancy can range from as young as 6 to as old as 77 years [22]. The age of onset for some types of familial cancers also depends on the generation affected, with progressively younger age of onset in successive generations, a phenomenon known as anticipation [23]. When confronted with the first generation affected, the age at diagnosis can be misleading.

Taking a comprehensive family history remains an essential tool when evaluating for the possibility of germline predisposition, but with families becoming smaller, the absence of a strong family history does not exclude the presence of germline mutations [24]. A pediatric study, analyzing 1120 children and adolescents with cancer found that only 40% of those with a PGV and available information on family history, had a positive family history for cancer [25]. In addition, several inherited predisposition syndromes can be caused by *de novo* germline mutations, ranging from as low as 0.3% in *BRCA1/2* related cancers to a more substantial portion of cases, as in *GATA2* deficiency syndromes [26–28]. Wlodarski et al. analysed 53 patients with *GATA2* deficiency syndromes that had familial information, of which 77% had no family history of myelodysplastic syndrome (MDS) or AML, and 9 out of the 11 (81%) patients whose parents also underwent sequencing had *de novo* germline mutations [29].

Molecular information

In some cases, the specific genetic variant found can provide clues as to its origin. For example, *DDX41* truncating mutations have only been found in the germline setting, with no somatic truncating variants identified to date [21]. In contrast, some genes can have identical variants present in both germline and somatic cases. This overlap and the challenges it presents can be seen in clear cell renal cell carcinoma, where mutations in the Von Hippel Lindau (*VHL*) gene are identified in more than 50% of tumor samples, but these same mutations can also be present as germline mutations in Von Hippel Lindau syndrome, a syndrome that predisposes to a variety of tumors including pheochromocytoma, hemangiomas and renal cell carcinoma, prompting the need for germline testing [30].

It is tempting to use VAF as an indicator of germline origin, with the rationale that any germline mutation would have a VAF close to 50% in heterozygous states, or 100% in homozygous states. However, this is overly simplistic and can lead to erroneous assumptions. In particular, we advise caution for clinicians who disclose results from commercial hematologic malignancy panels, as some of these companies use a dangerous practice of relying on the VAF to assign germline status. This strategy will not account for structurally modifying events, such as loss of heterozygosity and copy number variation, which can alter the VAF. There are reports in which using VAF alone only successfully distinguished germline from somatic variants in 48% of cases [3]. In contrast, one study did not find any germline mutations with a VAF less than 40% in a cohort of paired tumor-normal samples [31].

Some approaches attempt to use bioinformatic algorithms to distinguish between germline and somatic variants by modeling the expected allele frequencies of germline, somatic, and subclonal mutations using only mutational allelic fraction and sample purity [32]. Despite recognized limitations of this approach, such as the need for an accurate assessment of tumor versus normal tissue content of the sample, Sun et al. were able to distinguish between germline and somatic status in 85% of variants with an accuracy of 95–99% [32]. Other bioinformatic strategies try to differentiate between germline and somatic variants by using a virtual normal for comparison, composed of a pool of sequencing from a large number of unrelated healthy individuals, rather than a matched normal sample from the same individual [33]. This approach relies on the fact that most variants are common in the general population. This method has been found to filter out the majority of germline variants (96–99%) successfully within tumor samples [33]. The major limitation of this method, however, is that it will miss calling PGVs that are very rare in the general population, as these will likely not be present in the pool of normal sequences.

Paired tumor-normal sequencing

As was used in the majority of the studies reviewed in the "Frequency of Incidental Germline Findings" section, paired tumor-normal sequencing is thought to be the best way to differentiate between somatic and germline mutations found in tumor specimens [34]. Identical variants found in both the tumor and normal tissue are generally considered to be germline, whereas those isolated to the tumor are called somatic. For this method to be reliable, however, the choice of "normal" tissue plays a vital role. DNA extracted from peripheral blood is commonly used as the comparative normal tissue when paired tumor-germline sequencing is conducted for solid tumors. However, in the case of hematologic malignancies, peripheral blood cannot be used for this purpose, because the hematopoietic cells are the tumor cells and therefore contain both germline and tumor-associated mutations. The evidence for the ideal type of germline tissue in this setting is scarce. One study suggested that buccal swabs optimized for recovery of epithelial cells and T cells can be used, but the preferred germline source of DNA for hematological malignancies at the moment is cultured skin fibroblasts [34,35]. This approach minimizes blood contamination, however, it normally requires 3–4 weeks to allow the fibroblasts to expand to sufficient numbers for DNA extraction. Although it can be considered the gold standard, paired tumor-normal tissue sequencing is not always practical or feasible due to time, financial, and technical limitations.

We have developed a simplified flow chart as a guide to identifying germline variants from tumor-based sequencing (Fig. 1).

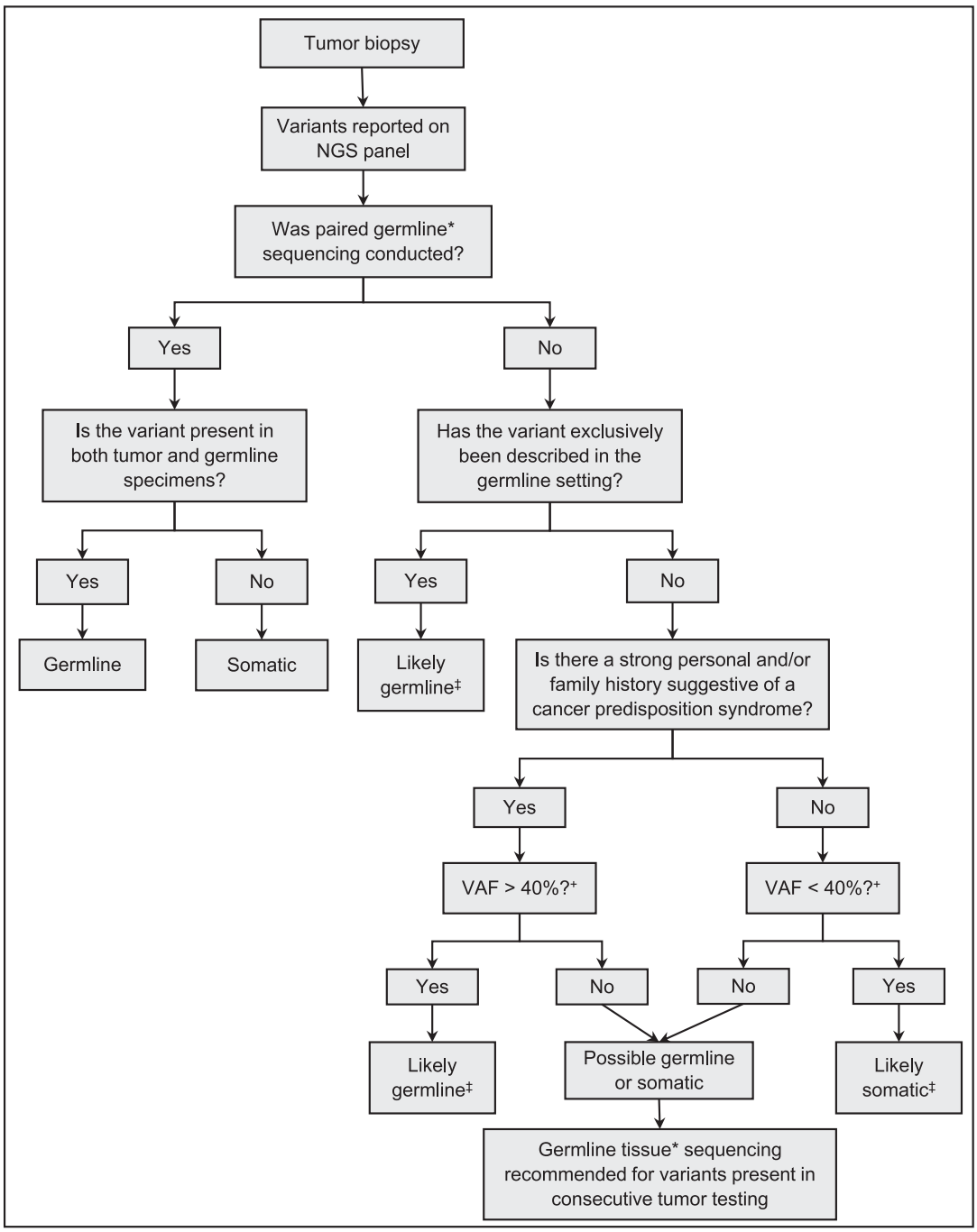

Fig. 1 How to differentiate between somatic and germline variants when variants are called on tumor-based sequencing. NGS, next generation sequencing; VAF, variant allele fraction. *Cultured skin fibroblasts are recommended as the source of germline DNA to eliminate possible contamination with blood and incidental detection of clonal hematopoiesis. +VAF should be interpreted with caution as it can be altered by structurally modifying events, such as loss of heterozygosity and uniparental disomy. The cut off of 40% was selected based on results from our group's previous study, which found no germline variants with a VAF under 40% [11]. ‡Germline tissue sequencing is recommended to confirm the variant status of "likely germline" or "likely somatic" variants.

How to report incidental germline findings

When a germline variant is found incidentally on tumor-based testing, we recommend following the standards and guidelines for the interpretation and reporting of sequence variants in cancer, a joint consensus recommendation by the Association for Molecular Pathology (AMP), American Society of Clinical Oncology (ASCO), and College of American Pathologists (CAP) [36]. This AMP/ASCO/CAP guideline recommends reporting incidentally found PGVs that have known clinical impact and/or are associated with a hereditary cancer syndrome for which established guidelines for clinical surveillance exist. For clinical exome or genome sequencing the ACMG includes a list of 59 medically actionable genes (Table 2), for which they

Table 2 List of 59 genes recommended by the ACMG for return of secondary findings in clinical exome or genome sequencing. Approximately half of these genes are associated with cancer predisposition syndromes and are often included in 'somatic' panels.

Disorder/Syndrome	Gene
Hereditary breast and ovarian cancer	*BRCA1, BRCA2*
Li–Fraumeni Syndrome	*TP53*
Peutz–Jeghers syndrome	*STK11*
Lynch syndrome	*MLH1, MSH2, MSH6, PMS2*
Familial adenomatous polyposis	*APC*
MYH-associated polyposis; adenomas, multiple colorectal, FAP type 2; colorectal adenomatous polyposis, autosomal recessive, with pilomatricomas	*MUTYH*
Juvenile polyposis	*BMPR1A, SMAD4*
Von Hippel–Lindau syndrome	*VHL*
Multiple endocrine neoplasia type 1	*MEN1*
Multiple endocrine neoplasia type 2	*RET*
Familial medullary thyroid cancer	*RET*
PTEN hamartoma tumor syndrome	*PTEN*
Retinoblastoma	*RB1*
Hereditary paraganglioma-pheochromocytoma syndrome	*SDHD, SDHAF2, SDHC, SDHB*
Tuberous sclerosis complex	*TSC1, TSC2*
WT1-related Wilms tumor	*WT1*
Neurofibromatosis type 2	*NF2*
Ehlers–Danlos syndrome, vascular type	*COL3A1*
Marfan syndrome, Loeys-Dietz syndromes, and familial thoracic aortic aneurysms and dissections	*FBN1, TGFBR1, TGFBR2, SMAD3, ACTA2, MYH11*
Hypertrophic cardiomyopathy, dilated cardiomyopathy	*MYBPC3, MYH7, TNNT2, TNNI3, TPM1, MYL3, ACTC1, PRKAG2, GLA, MYL2, LMNA*
Catecholaminergic polymorphic ventricular tachycardia	*RYR2*
Arrhythmogenic right ventricular cardiomyopathy	*PKP2, DSP, DSC2, TMEM43, DSG2*
Romano-Ward long-QT syndrome types 1, 2, and 3, Brugada syndrome	*KCNQ1, KCNH2, SCN5A*
Familial hypercholesterolemia	*LDLR, APOB, PCSK9*
Wilson disease	*ATP7B*
Ornithine transcarbamylase deficiency	*OTC*
Malignant hyperthermia susceptibility	*RYR1, CACNA1S*

Adapted from Kalia et al. [37].

Table 3 Suggested list of genes to report for return of secondary findings from tumor-based sequencing that confer increased risk of myeloid neoplasms.

Disorder/Syndrome	Gene
Familial MDS/AML	***RUNX1, GATA2, ETV6, CEBPA, DDX41, ANKRD26,*** *SRP72, SAMD9, SAMD9L*
Inherited bone marrow failure syndromes with germline predisposition to myeloid neoplasms	***TERT, TERC, FANC* genes, *DKC1, ELANE, HAX1, GFI1***
Inherited syndromes associated with myeloid neoplasms	*TP53, PTPN11, CBL, KRAS, NF1, BLM, ATG2B/GSKIP, BRCA1,* ***BRCA2***

AML, acute myeloid leukemia; MDS, myelodysplastic syndrome. Bolded genes are included in both the 2016 WHO classification of hematologic malignancies and the 2017 NCCN MDS clinical practice guideline [12,40].

recommend reporting incidental or secondary germline findings [37]. Approximately half of these genes are known cancer predisposition genes and are often included in 'somatic' panels. The AMP/ASCO/CAP also recommends disclosure of PGVs found within these 59 genes when they are identified through paired tumor-normal sequencing [36].

No such guidelines or lists exist for the reporting of incidentally found germline variants associated with hematologic malignancies. However, reporting genes included in the newly incorporated sections on germline predisposition to myeloid malignancies in the 2016 World Health Organization (WHO) classification of hematologic malignancies and the 2017 NCCN MDS clinical practice guideline as well as *SAMD9* and *SAMD9L*, more recently implicated in familial MDS, would be reasonable (Table 3) [12,38–40]. More comprehensive lists and details on genes associated with germline predisposition to hematologic malignancies can be found elsewhere [13,41–47].

Clonal Hematopoiesis: another confounding factor

When it comes to differentiating between somatic and germline variants on tumor-based NGS, the possibility of detecting CH rather than somatic tumor variants must also be considered. CH describes the clonal expansion of blood cells derived from a hematopoietic stem or progenitor cell that has acquired one or more somatic mutations [48]. The term clonal hematopoiesis of indeterminate potential (CHIP) is used when the somatic mutation occurs in a gene known to be associated with hematologic malignancy, but diagnostic criteria for a hematologic malignancy are not fulfilled [49]. When a somatic mutation is present in a patient with unexplained cytopenias who does not otherwise meet diagnostic criteria for a hematologic malignancy, the term clonal cytopenias of undetermined significance (CCUS) is used [50]. Both CHIP and CCUS are encompassed under the broader heading of CH. CH is found in up to 10% of persons over age 65 years and 1% of those under the age of 50 years [51].

The potential for incidental detection of CH from tumor-based NGS was first brought to light with the identification of *JAK2* V617F, a variant commonly found in myeloproliferative neoplasms, non-small cell lung cancer, and duodenal cancer [52,53]. Lee et al. reported a case of a *JAK2* V617F variant mistakenly assigned as a somatic duodenal cancer mutation by panel tumor-based NGS in a patient with polycythemia vera [52]. The mutation was initially found to be present in both tumor tissue, which contains a mix of tumor, germline tissue, and blood cells and in a buccal swab specimen, which contains germline DNA, but is often contaminated by blood cells. Subsequent testing in normal tissue and fingernail DNA, two sources of germline DNA, failed to detect this mutation, indicating that it was somatic rather than germline and likely present in the tumor and buccal cells, because of contamination with blood cells, as opposed to a tumor-derived somatic mutation.

The concept that the presence of CH can confound tumor-only sequencing results was further examined by Ptashkin et al. [54]. They analyzed targeted NGS data of paired blood and tumor samples from 17,469 patients and identified 7608 variants in 396 genes from 4628 patients. Among these variants, 1075 of them from 912 patients, were derived from CH, accounting for 14.1% of all identified variants within 5.2% of the evaluated population. CH events were most common in *DNMT3A, TET2*, and *PPM1D*. By comparing to a tumor-only variant calling pipeline, the authors inferred that 98.7% of these CH alterations would have been called tumor-associated somatic variants, highlighting how false assignment of variants can occur when tumor-only sequencing is used, and results are not critically evaluated. The detection of CH may be due to the presence of infiltrating leukocytes within the tumor specimens, contamination with whole blood during the biopsy, and/or directly sequencing hematopoietic cells in the case of hematologic malignancies and can further confuse the results of tumor-based sequencing.

Return to the case

Armed with the knowledge presented above, you attempt to determine the likelihood of the three different possible origins for this *TP53* (c.743G>A, p.R248Q) variant: germline, contaminating CLL cells, or CH. This particular variant has been found in both germline and somatic settings, and therefore, the allele itself cannot be used to differentiate between the two [55,56]. The young age of presentation with breast cancer and the presence of multiple malignancies in the same individual are suggestive, but not pathognomonic, of a germline variant. Further detailed family history was taken, revealing that the patient's mother had breast cancer at the age of 35 and passed away from AML in her 50′s; a maternal uncle had been diagnosed with a soft-tissue sarcoma at age 23, lending further suspicion towards a germline origin to this variant and away from contamination with CLL cells or CH as being the culprit. To confirm if it was a germline variant, a skin biopsy was taken from the patient, and DNA was extracted from cultured skin fibroblasts. The same *TP53* (c.743G>A, p.R248Q) variant, at a VAF of 53%, was present, confirming its germline origin and hence the diagnosis of LFS. Further evaluation of the sequencing done on the patient's Fallopian tube tumor found that the high VAF was due to a loss of heterozygosity event within the tumor. LFS results from pathogenic germline *TP53* mutations and confers high risk of developing cancer from multiple different organ systems starting at a young age, with 50% developing cancer by the age of 30 years [17,57]. Upon your suggestion, the patient was referred for genetic counselling. She opted to have her remaining breast tissue removed, had her remaining fallopian tube and ovary excised, and initiated enhanced colorectal cancer screening in addition to close clinical follow-up. She notified her family members and encouraged them to have their own testing and genetic counselling.

Conclusions

Tumor or 'somatic' mutation NGS panels are commonly performed with the goal of identifying driver mutations that provide insight into disease biology, prognosis, as well as uncovering possible actionable variants that can be targeted therapeutically. However, tumor-only sequencing not only reveals the somatic mutation profile of the tumor, but also any germline variants that the patient may carry and/or the presence of clonal hematopoiesis due to contamination with blood or leukocyte infiltration, and it can be difficult to distinguish among these. Numerous studies, spanning both solid and hematologic malignancies, have proven that these incidental or secondary findings are not rare events as once thought and should not be ignored. Clinicians ordering these tests must be aware of this possibility and be properly equipped to interpret the results and appropriately council their patients. Rather than 'somatic' mutation panels, the name of these panels should reflect their ability to identify tumor-derived somatic mutations, germline variants, and even clonal hematopoiesis.

Declarations of interest

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References

[1] Ngeow J, Eng C. Precision medicine in heritable cancer: when somatic tumour testing and germline mutations meet. NPJ Genom Med 2016;1:15006. doi:10.1038/npjgenmed.2015.6.

[2] Kilpivaara O, Aaltonen LA. Sequencing and the contribution of germline variants. Science 2013;339:1559–62. doi:10.1126/science.1233899.

[3] Jones S, Anagnostou V, Lytle K, Parpart-Li S, Nesselbush M, Riley DR, et al. Personalized genomic analyses for cancer mutation discovery and interpretation. Sci Transl Med 2015;7 283ra53. doi:10.1126/scitranslmed.aaa7161.

[4] Seifert BA, O'Daniel JM, Amin K, Marchuk DS, Patel NM, Parker JS, et al. Germline analysis from tumor-germline sequencing dyads to identify clinically actionable secondary findings. Clin Cancer Res 2016;22:4087–94. doi:10.1158/1078-0432.CCR-16-0015.

[5] Meric-Bernstam F, Brusco L, Daniels M, Wathoo C, Bailey AM, Strong L, et al. Incidental germline variants in 1000 advanced cancers on a prospective somatic genomic profiling protocol. Ann Oncol Off J Eur Soc Med Oncol 2016;27:795–800. doi:10.1093/annonc/mdw018.

[6] Catenacci DVT, Amico AL, Nielsen SM, Geynisman DM, Rambo B, Carey GB, et al. Tumor genome analysis includes germline genome: are we ready for surprises? Int J Cancer 2015;136:1559–67. doi:10.1002/ijc.29128.

[7] Slavin TP, Banks KC, Chudova D, Oxnard GR, Odegaard JI, Nagy RJ, et al. Identification of incidental germline mutations in patients with advanced solid tumors who underwent cell-free circulating tumor DNA sequencing. J Clin Oncol 2018;36:3459–65. doi:10.1200/JCO.18.00328.

[8] Schrader KA, Cheng DT, Joseph V, Prasad M, Walsh M, Zehir A, et al. Germline variants in targeted tumor sequencing using matched normal DNA. JAMA Oncol 2016;2:104–11. doi:10.1001/jamaoncol.2015.5208.

[9] Mandelker D, Zhang L, Kemel Y, Stadler ZK, Joseph V, Zehir A, 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;318:825–35. doi:10.1001/jama.2017.11137.

[10] Huang K-L, Mashl RJ, Plon SE, Chen F, Ding L. Pathogenic germline variants in 10,389 adult cancers. Cell 2018;173:355–70. doi:10.1016/j.cell.2018.03.039.

[11] Drazer MW, Kadri S, Sukhanova M, Patil SA, West AH, Feurstein S, et al. Prognostic tumor sequencing panels frequently identify germ line variants associated with hereditary hematopoietic malignancies. Blood Adv 2018;2:146–50. doi:10.1182/bloodadvances.2017013037.

[12] Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 2016;127:2391–405. doi:10.1182/blood-2016-03-643544.

[13] Godley LA, Shimamura A. Genetic predisposition to hematologic malignancies: management and surveillance. Blood 2017;130:424–32. doi:10.1182/blood-2017-02-735290.

[14] Tung NM, Garber JE. BRCA1/2 testing: therapeutic implications for breast cancer management. Br J Cancer 2018;119:141–52. doi:10.1038/s41416-018-0127-5.

[15] University of Chicago Hematopoietic Malignancies Cancer Risk Team TU of CHMCR How I diagnose and manage individuals at risk for inherited myeloid malignancies. Blood 2016;128:1800–13. doi:10.1182/blood-2016-05-670240.

[16] Raymond VM, Gray SW, Roychowdhury S, Joffe S, Chinnaiyan AM, Parsons DW, et al. Germline findings in tumor-only sequencing: points to consider for clinicians and laboratories. J Natl Cancer Inst 2016;108:1–5. doi:10.1093/jnci/djv351.

[17] McBride KA, Ballinger ML, Killick E, Kirk J, Tattersall MHN, Eeles RA, et al. Li-Fraumeni syndrome: cancer risk assessment and clinical management. Nat Rev Clin Oncol 2014;11:260–71. doi:10.1038/nrclinonc.2014.41.

[18] Xiao H, Shi J, Luo Y, Tan Y, He J, Xie W, et al. First report of multiple CEBPA mutations contributing to donor origin of leukemia relapse after allogeneic hematopoietic stem cell transplantation. Blood 2011;117:5257–60. doi:10.1182/blood-2010-12-326322.

[19] Edelmann J, Gribben JG. Managing patients with TP53-deficient chronic lymphocytic leukemia. J Oncol Pract 2017;13:371–7. doi:10.1200/JOP.2017.023291.

[20] Dietrich S, Pircher A, Endris V, Peyrade F, Wendtner C-M, Follows GA, et al. BRAF inhibition in hairy cell leukemia with low-dose vemurafenib. Blood 2016;127:2847–55. doi:10.1182/blood-2015-11-680074.

[21] Polprasert C, Schulze I, Sekeres MA, Makishima H, Przychodzen B, Hosono N, et al. Inherited and somatic defects in DDX41 in myeloid neoplasms. Cancer Cell 2015;27:658–70. doi:10.1016/j.ccell.2015.03.017.

[22] Sood R, Kamikubo Y, Liu P. Role of RUNX1 in hematological malignancies. Blood 2017;129:2070–82. doi:10.1182/blood-2016-10-687830.

[23] Vulliamy T, Marrone A, Szydlo R, Walne A, Mason PJ, Dokal I. Disease anticipation is associated with progressive telomere shortening in families with dyskeratosis congenita due to mutations in TERC. Nat Genet 2004;36:447–9. doi:10.1038/ng1346.

[24] Frank C, Fallah M, Sundquist J, Hemminki A, Hemminki K. Population landscape of familial cancer. Sci Rep 2015;5:1–8. doi:10.1038/srep12891.

[25] Zhang J, Walsh MF, Wu G, Edmonson MN, Gruber TA, Easton J, et al. Germline mutations in predisposition genes in pediatric cancer. N Engl J Med 2015;373:2336–46. doi:10.1056/NEJMoa1508054.

[26] Chompret A, Brugieres L, Ronsin M, Gardes M, Dessarps-Freichey F, Abel A, et al. P53 germline mutations in childhood cancers and cancer risk for carrier individuals. Br J Cancer 2000;82:1932–7. doi:10.1054/bjoc.2000.1167.

[27] Win AKO, Jenkins MA, Buchanan DD, Clendenning M, Young JP, Giles GG, et al. Determining the frequency of de novo germline mutations in DNA mismatch repair genes. J Med Genet 2011;48:530–4. doi:10.1136/jmedgenet-2011-100082.

[28] Golmard L, Delnatte C, Laugé A, Moncoutier V, Lefol C, Abidallah K, et al. Breast and ovarian cancer predisposition due to de novo BRCA1 and BRCA2 mutations. Oncogene 2016;35:1324–7. doi:10.1038/onc.2015.181.

[29] Wlodarski MW, Hirabayashi S, Pastor V, Stary J, Hasle H, Masetti R, et al. Prevalence, clinical characteristics, and prognosis of GATA2-related myelodysplastic syndromes in children and adolescents. Blood 2016;127:1387–97. doi:10.1182/blood-2015-09-669937.

[30] Brugarolas J. Molecular genetics of clear-cell renal cell carcinoma. J Clin Oncol 2014;32:1968–76. doi:10.1200/JCO.2012.45.2003.

[31] Drazer MW, Kadri S, Sukhanova M, Patil SA, West AH, Feurstein S, et al. Prognostic tumor sequencing panels frequently identify germ line variants associated with hereditary hematopoietic malignancies. Blood Adv 2018;2:146–50. doi:10.1182/bloodadvances.2017013037.

[32] Sun JX, He Y, Sanford E, Montesion M, Frampton GM, Vignot S, et al. A computational approach to distinguish somatic

vs. germline origin of genomic alterations from deep sequencing of cancer specimens without a matched normal. PLoS Pathog 2018;14:1–13. doi:10.1371/journal.pcbi.1005965.
[33] Hiltemann S, Jenster G, Trapman J, Van Der Spek P, Stubbs A. Discriminating somatic and germline mutations in tumor DNA samples without matching normals. Genome Res 2015;25:1382–90. doi:10.1101/gr.183053.114.
[34] Churpek JE, Godley LA. How I diagnose and manage individuals at risk for inherited myeloid malignancies. Blood 2016;128:1800–13. doi:10.1182/blood-2016-05-670240.
[35] Padron E, Ball MC, Teer JK, Painter JS, Yoder SJ, Zhang C, et al. Germ line tissues for optimal detection of somatic variants in myelodysplastic syndromes. Blood 2018;131:2402–5. doi:10.1182/blood-2018-01-827881.
[36] Li MM, Datto M, Duncavage EJ, Kulkarni S, Lindeman NI, Roy S, 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;19:4–23. doi:10.1016/j.jmoldx.2016.10.002.
[37] Kalia SS, Adelman K, Bale SJ, Chung WK, Eng C, Evans JP, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. Genet Med 2017;19:249–55. doi:10.1038/gim.2016.190.
[38] Narumi S, Amano N, Ishii T, Katsumata N, Muroya K, Adachi M, et al. SAMD9 mutations cause a novel multisystem disorder, MIRAGE syndrome and are associated with loss of chromosome 7. Nat Genet 2016;48:792–7. doi:10.1038/ng.3569.
[39] Tesi B, Davidsson J, Voss M, Rahikkala E, Holmes TD, Chiang SCC, et al. Gain-of-function SAMD9L mutations cause a syndrome of cytopenia, immunodeficiency, MDS, and neurological symptoms. Blood 2017;129:2266–79. doi:10.1182/blood-2016-10-743302.
[40] Greenberg PL, Stone RM, Al-Kali A, Barta SK, Bejar R, Bennett JM, et al. Myelodysplastic syndromes, Version 2.2017, NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw 2017;15:60–87. doi:10.6004/JNCCN.2017.0007.
[41] Petrucelli N., Daly M.B., Pal T. BRCA1- and BRCA2-associated hereditary breast and ovarian cancer. GeneReviews® [Internet] 2016. http://www.ncbi.nlm.nih.gov/pubmed/20301425 (accessed 5 February 2019).
[42] Noetzli L, Lo RW, Lee-Sherick AB, Callaghan M, Noris P, Savoia A, et al. Germline mutations in ETV6 are associated with thrombocytopenia, red cell macrocytosis and predisposition to lymphoblastic leukemia. Nat Genet 2015;47:535–8. doi:10.1038/ng.3253.
[43] Kirwan M, Walne AJ, Plagnol V, Velangi M, Ho A, Hossain U, et al. Exome sequencing identifies autosomal-dominant SRP72 mutations associated with familial aplasia and myelodysplasia. Am J Hum Genet 2012;90:888–92. doi:10.1016/J.AJHG.2012.03.020.
[44] Feurstein S, Drazer MW, Godley LA. Genetic predisposition to leukemia and other hematologic malignancies. Semin Oncol 2016;43:598–608. doi:10.1053/J.SEMINONCOL.2016.10.003.
[45] Ballew BJ, Savage SA. Updates on the biology and management of dyskeratosis congenita and related telomere biology disorders. Expert Rev Hematol 2013;6:327–37. doi:10.1586/ehm.13.23.
[46] Hays L, Frohnmayer D, Frohnmayer L, Guinan E, Kennedy T, Larsen K, editors. Fanconi Anemia: guidelines for diagnosis and management. Eds.Forth Ed.. Fanconi Anemia Research Fund, Inc.; 2014.
[47] Dror Y, Donadieu J, Koglmeier J, Dodge J, Toiviainen-Salo S, Makitie O, et al. Draft consensus guidelines for diagnosis and treatment of Shwachman-Diamond syndrome. Ann N Y Acad Sci 2011;1242:40–55. doi:10.1111/j.1749-6632.2011.06349.x.
[48] Bowman RL, Busque L, Levine RL. Cell stem cell review clonal hematopoiesis and evolution to hematopoietic malignancies. Stem Cell 2018;22:157–70. doi:10.1016/j.stem.2018.01.011.
[49] Steensma DP, Bejar R, Jaiswal S, Lindsley RC, Sekeres MA, Hasserjian RP, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. Blood 2015;126:9–16. doi:10.1182/blood-2015-03-631747.
[50] Kwok B, Hall JM, Witte JS, Xu Y, Reddy P, Lin K, et al. MDS-associated somatic mutations and clonal hematopoiesis are common in idiopathic cytopenias of undetermined significance. Blood 2015;126:2355–61. doi:10.1182/blood-2015-08-667063.
[51] Genovese G, Kähler AK, Handsaker RE, Lindberg J, Rose SA, Bakhoum SF, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N Engl J Med 2014;371:2477–87. doi:10.1056/NEJMoa1409405.
[52] Lee J, Axilbund J, Dalton WB, Laheru D, Watkins S, Chu D, et al. A polycythemia vera JAK2 mutation masquerading as a duodenal cancer mutation. J Natl Compr Canc Netw 2016;14:1495–8.
[53] Li SD, Ma M, Li H, Waluszko A, Sidorenko T, Schadt EE, et al. Cancer gene profiling in non-small cell lung cancers reveals activating mutations in JAK2 and JAK3 with therapeutic implications. Genome Med 2017;9:89. doi:10.1186/s13073-017-0478-1.
[54] Ptashkin RN, Mandelker DL, Coombs CC, Bolton K, Yelskaya Z, Hyman DM, et al. Prevalence of clonal hematopoiesis mutations in tumor-only clinical genomic profiling of solid tumors. JAMA Oncol 2018;4:1589–93. doi:10.1001/jamaoncol.2018.2297.
[55] Amadou A, Waddington Achatz MI, Hainaut P. Revisiting tumor patterns and penetrance in germline TP53 mutation carriers: temporal phases of Li-Fraumeni syndrome. Curr Opin Oncol 2018;30:23–9. doi:10.1097/CCO.0000000000000423.
[56] Tate J.G., Bamford S., Jubb H.C., Sondka Z., Beare D.M., Bindal N., et al. COSMIC : the catalogue of somatic mutations in cancer 2019;47:941–7. doi:10.1093/nar/gky1015.
[57] Schneider K., Zelley K., Nichols K.E., Garber J. Li-Fraumeni syndrome. GeneReviews® [Internet] 2013. https://www.ncbi.nlm.nih.gov/books/NBK1311/ (accessed 6 February 2019).