Germline Sequencing Improves Tumor-Only Sequencing Interpretation in a Precision Genomic Study of Patients With Pediatric Solid Tumor

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PURPOSE Molecular tumor profiling is becoming a routine part of clinical cancer care, typically involving tumor-only panel testing without matched germline. We hypothesized that integrated germline sequencing could improve clinical interpretation and enhance the identification of germline variants with significant hereditary risks.

MATERIALS AND METHODS Tumors from pediatric patients with high-risk, extracranial solid malignancies were sequenced with a targeted panel of cancer-associated genes. Later, germline DNA was analyzed for a subset of these genes. We performed a post hoc analysis to identify how an integrated analysis of tumor and germline data would improve clinical interpretation.

RESULTS One hundred sixty participants with both tumor-only and germline sequencing reports were eligible for this analysis. Germline sequencing identified 38 pathogenic or likely pathogenic variants among 35 (22%) patients. Twenty-five (66%) of these were included in the tumor sequencing report. The remaining germline pathogenic or likely pathogenic variants were single-nucleotide variants filtered out of tumor-only analysis because of population frequency or copy-number variation masked by additional copy-number changes in the tumor. In tumor-only sequencing, 308 of 434 (71%) single-nucleotide variants reported were present in the germline, including 31% with suggested clinical utility. Finally, we provide further evidence that the variant allele fraction from tumor-only sequencing is insufficient to differentiate somatic from germline events.

CONCLUSION A paired approach to analyzing tumor and germline sequencing data would be expected to improve the efficiency and accuracy of distinguishing somatic mutations and germline variants, thereby facilitating the process of variant curation and therapeutic interpretation for somatic reports, as well as the identification of variants associated with germline cancer predisposition.

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BACKGROUND Precision medicine among oncology patients has been accelerated by the widespread use of molecular tumor profiling. In pediatric oncology, tumor profiling can refine a histologic diagnosis, inform prognosis, and identify variants that predict response to targeted therapies.1,2 Many academic and commercial assays are designed to detect variants in a panel of genes commonly associated with cancer. Although some groups use matched paired tumor-normal sequencing,3,6 the College of American Pathologists reported that 90% of clinical next-generation sequencing (NGS) laboratories perform tumor-only testing.7 For tumor-only assays, analytic pipelines aim to reduce the number of germline variants in the output, often by filtering out variants recurrently reported in genomic population databases. Rare germline variants, however, can still be included in reports.6,8-10 By focusing on somatic events, these computational filters may also mask germline variants associated with cancer predisposition, as demonstrated in prior studies.5,10,11 To further refine tumor variant calls, sequencing data from tumor-only panels are often
CONTEXT

Key Objective
Does the addition of germline sequencing affect the interpretation of tumor-only sequencing data in a therapeutic study of pediatric high-risk solid tumors?

Knowledge Generated
Clinical reports with therapeutic recommendations on the basis of tumor-only sequencing analyses can include a significant number of genetic variants that cannot be definitively classified as germline or somatic on the basis of variant allele fraction or expert curation. In addition, tumor sequencing alone may not adequately identify all germline variants with clinical significance.

Relevance
Integrated tumor-germline genetic profiling would improve the classification of somatic mutations in cancer, decrease the amount of time and resources spent curating variants, and increase the identification of germline variants with clinical significance. This has particular relevance for the clinical care of individuals and families with cancer predisposition.

reviewed and interpreted by molecular pathologists or geneticists who may identify the possibility that certain variants are germline in their reports. Together, these approaches are designed to improve the accuracy of variant reporting to refine diagnoses, aid in treatment decisions, and refer patients and families to genetic counseling when indicated. However, direct assessment of the effectiveness of computational filters, expert review, and the potential added value of comparative or paired tumor and germline analysis has not been extensively examined in pediatric cancer cohorts.

In this study, we analyzed a subset of patients with childhood solid malignancies enrolled in a genomic precision medicine trial, Genomic Assessment Improves Novel Therapy (GAIN) consortium study (ClinicalTrials.gov Identifier: NCT02520713). Tumor samples underwent tumor-only profiling followed by germline profiling, with separate reports provided to clinicians. Given the sequential analysis of tumor followed by germline sequencing, data from this cohort provide a unique opportunity for a post hoc analysis to compare how the addition of germline sequencing may affect clinical variant interpretation and clinical recommendations. We hypothesized that tumor-only sequencing could result in the inability to definitively distinguish germline from somatic variants, potentially complicating treatment decisions and missing opportunities to identify hereditary cancer risk in patients and their families.

MATERIALS AND METHODS

GAIN Trial Enrollment and Selection of Patients for Study
Patients were identified among those enrolled on the GAIN Consortium trial approved by the Institutional Review Boards at Dana-Farber Cancer Institute and participating sites. Eligible patients had a high-risk (defined as an expected 2-year progression free survival of 50% or less) newly diagnosed or relapsed or refractory solid malignancy outside of the central nervous system with an age at initial diagnosis of 30 years or younger. Written informed consent was obtained for all study participants. Tumor samples were acquired from archival material collected as part of routine clinical care, and peripheral blood was obtained during a clinical blood draw. Physicians and research staff provided pathologic diagnosis and demographic information, including participant-reported race and ethnicity. Individuals from the GAIN trial were eligible for inclusion in this analysis if both tumor and germline profiling results, as well as completed clinical interpretation and reports, were available for analysis by April 2019.

Tumor Sequencing and Variant Curation
Tumor samples were sequenced by the Center for Advanced Molecular Diagnostics at Brigham and Women’s Hospital using a custom hybrid capture sequencing assay, OncoPanel, targeting 300 genes (version POPv2) or 447 genes (version POPv3). Briefly, extracted DNA underwent next-generation sequencing of targeted genes using the TruSeq LT library preparation kit (Illumina, San Diego, CA), a custom RNA bait set (Agilent SureSelect, Agilent, Santa Clara, CA), and the Illumina HiSeq2500. Analysis included the detection of sequence variants, copy-number alterations, and structural variants, as previously described. The lower limit of detection for sequence variants was 10% allelic fraction at 50x coverage. Likely polymorphisms and artifacts were filtered by comparing variant calls to both a panel of normal samples and in-batch normal controls, as well as those found in the NHLBI Exome Sequencing Project (ESP) and/or gnomAD databases at > 0.1% frequency in any subpopulation. Variants flagged for filtering that were present in Cosmic at least twice were subsequently rescued. Variants were interpreted and reported by a molecular pathologist according to guidelines recommended by the Association for Molecular Pathology, College of American Pathologists, and American Society of Clinical Oncology17 within a 5-tier schema: Variants in tiers
Clinical Interpretation

The GAIN study aims to evaluate whether tumor sequencing increases the use of molecularly targeted therapies and whether those therapies are associated with treatment responses. Such individualized cancer therapy (iCat) recommendations were made on the basis of peer-reviewed literature and, in cases with weak or conflicting evidence, consensus opinion from an expert panel. Treatment recommendations were tiered (separate from the variant tiers described above) on the basis of the strength of the evidence supporting potential response to a specific therapy.1 The iCat recommendation, including a comment about possible germline origin if appropriate, was communicated in a written report.

Germline Sequencing and Variant Curation

Germline profiling was performed on DNA extracted from peripheral blood samples using OncoPanel version POPv3. Analysis was informatically restricted to 147 genes known to be associated with increased cancer risk, as previously described.18 Germline single-nucleotide variants (SNVs) and copy-number variants (CNVs) were classified as pathogenic (P), likely pathogenic (LP), variant of uncertain significance (VUS), likely benign (LB), or benign (B) by a molecular pathologist according to guidelines for the interpretation of germline variants, as recommended by the American College of Medical Genetics and the Association for Molecular Pathology.19 Although the B/LB variants were not included in the pathologists’ reports, these were available for review for the purposes of this study.

Comparison of Tumor-Only and Germline-Only Reports With Integrated Data Analysis

We compared the variant lists from tumor-only reports to those of germline reports and the B/LB germline variant list. We assessed the tumor-only and germline-only sequencing results for (1) the percentage of variants in the tumor-only report that were determined to be germline (including P/LP, VUS, and B/LB), (2) the proportion of P/LP germline variants present in the tumor-only report, and (3) the variant allele fractions (VAFs) of true somatic events compared with germline variants in the tumor-only report and germline-only report using descriptive analyses.

For each germline P/LP variant identified, tumor sequencing data were examined for the presence of a second alteration in the same gene within the tumor, including loss-of-function SNVs, deletions, or copy-neutral loss of heterozygosity (CN-LOH). In addition, for the P/LP variants not present on the tumor-only sequencing report, we reviewed the tumor sequencing data, including filtered variants, within the laboratory variant database.

RESULTS

Germline Variants Identified in the Study Cohort

One hundred and sixty participants enrolled in the GAIN trial met the eligibility criteria for this study (Fig 1). In this cohort, the average age at diagnosis was 12 years (range, 1 month to 27 years). There were 42 different cancer diagnoses, with osteosarcoma being the most frequent (Table 1).

| Table 1. Baseline Characteristics for the Cohort |
| Variable | Mean (SD) or No. (%) |
|------------------|-------------------|
| Age at diagnosis | 12 (5.8) |
| Sex, No. (%) | |
| Female | 75 (47) |
| Male | 85 (53) |
| Race, No. (%) | |
| White | 113 (71) |
| Black or African American | 14 (9) |
| Asian | 9 (6) |
| More than one race | 1 (1) |
| Native Hawaiian/Other Pacific Islander | 1 (1) |
| Unknown | 3 (2) |
| Other | 19 (12) |
| Ethnicity, No. (%) | |
| Hispanic or Latino | 17 (11) |
| Non-Hispanic | 127 (79) |
| Unknown | 16 (10) |
| Cancer diagnosis type, No. (%) | |
| Osteosarcoma | 58 (36) |
| Rhabdomyosarcoma | 18 (11) |
| Ewing sarcoma | 13 (8) |
| Other sarcoma | 29 (18) |
| Renal tumor | 11 (7) |
| Neuroblastoma | 12 (8) |
| Liver tumor | 4 (3) |
| Carcinoma | 4 (3) |
| Other | 11 (7) |

Abbreviation: SD, standard deviation.
being the most common (n = 12, Appendix Table A1). Ten (26%) of the P/LP variants were in genes known to be associated with the patient’s cancer diagnosis. Pathogenic variants in genes not known to be clearly associated with the patient’s diagnosis included BRCA1 in a child with neuroblastoma, MITF in a child with Ewing sarcoma, and FAM175A variant in a child with osteosarcoma (Appendix Table A1).

Of the 17 germline P/LP variants in autosomal dominant cancer risk genes, we identified a second alteration within the same gene in 9 (53%) of the associated tumors (Fig 2A, Appendix Table A1). Four patients with DICER1 variants had a second DICER1 hotspot SNV in the tumor, three patients had a somatic deletion of the other allele, and two had CN-LOH associated with overrepresentation of the variant allele. None of the tumors from patients who were carriers of an autosomal recessive cancer risk gene had a second alteration identified.

Variants Identified in Tumor-Only Sequencing Are Frequently Germline in Origin

To study the potential germline etiology of variants included in tumor profiling reports, we compared tumor-only and germline-only sequencing reports from the 160 patients included in this study (Fig 1). For 9 cases using OncoPanel version POPv2, our analysis was restricted to 86 genes analyzed by both tumor and germline pipelines, whereas our analysis included 147 genes for 151 cases profiled with POPv3. Four hundred thirty-four tumor SNVs (48 tier 1-2, 43 tier 3, 343 tier 4), 492 germline P/LP/VUS SNVs, and 332 germline B/LB SNVs were identified.

Of the 434 SNVs identified by tumor sequencing, 285 (66%) were reported as P/LP/VUS in the germline sequencing report and 23 (5%) were classified as B/LB variants by the molecular pathologist. Only 126 variants (29%) were present solely in the tumor sequencing data, and thus determined to be of somatic origin (Fig 3A).

Next, we investigated how many of the P/LP germline variants had been noted as potentially germline in the tumor sequencing report. Of the 285 P/LP/VUS germline SNVs present in the tumor-only sequencing report, 23 were P/LP germline variants occurring in nine autosomal dominant genes (APC, CHEK2, DICER1, FAM175A, RB1, SDHA, SMARCA4, TP53, and UROD) and seven autosomal recessive genes (BLM, DOCK8, GBA, MUTYH, SERPINA1, WRN, and XPA). All 23 variants, except one autosomal dominant LP variant in UROD, were noted as possibly germline. In 18 of 23 cases, the tumor-only sequencing reports also emphasized that the sequencing assay could not distinguish between germline or somatic variants, with 15 of these adding that genetic counseling may be helpful, clinically indicated, or specifically recommended. Two referenced previous clinical germline testing that had identified the same variant.
Of 148 SNVs reported as possibly germline in the tumor-only report, 52 (35%) were not present in the patient’s germline report or B/LB variant list. These variants were also located within all four clinical tiers in the tumor report (Fig 3C).

Finally, we found that two of the three P/LP CNVs identified from germline sequencing were also reported as variants in tumor-only sequencing (ELANE and FANCA). Neither of these was flagged as possibly germline.

**VAF Is Not Sufficient to Clarify Germline or Somatic Origin**

We next investigated to what degree the allelic fraction of variants from tumor-only sequencing could help predict which variants were of germline origin. The median VAF for confirmed somatic variants and confirmed germline variants within the tumor-only sequencing was 35.6% (range 3.5%-100%) and 48.3% (range 3.94%-95.3%), respectively (Fig 4). Although the median VAF was significantly different between the two groups ($P < .0001$, Mann-Whitney test), we observed considerable overlap, as 35.1% of true somatic mutations had VAFs inside the informally accepted range of 40%-60% that, in our experience, is frequently discussed as indicating a possible germline origin. Conversely, we found that 30.7% of true germline variants had a VAF that was either > 60% or below 40% in the tumor-only sequencing data, of which only 35% had a called copy-number variant of the gene (Appendix Fig A1). Interestingly, 94% of germline variants identified from a normal blood sample were found to have VAFs between 40% and 60% (Fig 4).

**Impact of Germline Variants on Therapeutic Recommendations**

Treatment recommendations were made on the basis of 64 SNVs within tumor-only sequencing for 53 of the 160 cases included in this study, and 46 were made on the basis of genes present on both somatic and germline sequencing panels. Eleven (24%) of these 46 recommendations were on the basis of variants of confirmed germline origin, including seven P/LP SNVs within the genes CHEK2, FAM175A, SDHA, SMARCA4, and TP53, and four VUS in AKT1, NBN, TP53, and TSC2 (Table 2). Treatment recommendations noted that seven of these 11 variants may be of germline etiology and identified all four of the VUS alterations as variants of uncertain significance.

**Tumor-Only Sequencing May Exclude Significant Germline Variants**

Twelve of the 35 germline P/LP SNVs and one of the three germline P/LP CNVs were not reported (Appendix Table A1). Three of these were in autosomal dominant cancer risk genes (APC, BRCA1, and MTF).

In examining the tumor-only BAM files, all 12 SNVs were present in the aligned sequencing data but were filtered out by the somatic analytic pipeline because of their high population allele frequencies. The pathogenic germline CNV in BRCA1 was masked in the tumor-only sequencing by an overlapping somatic copy-gain in 17q.

**DISCUSSION**

An important goal of precision medicine in cancer is to improve patient outcomes through a deep understanding of the potential targetable vulnerabilities present in the tumor. At present, tumor-only NGS panels are the most widely used modality to identify biomarkers of response to molecularly targeted therapies. We sought to identify the added benefits of paired tumor-germline sequencing in a pediatric cancer cohort using the availability of sequential tumor and germline sequencing as a proxy. In our high-

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**FIG 2.** Germline pathogenic and likely pathogenic variants. (A) There were 18 heterozygous P/LP variants detected across 12 different autosomal dominant cancer risk genes. Nine of the tumors had a second mutation within the same gene (*). (B) There were 20 heterozygous P/LP variants detected across 10 different autosomal recessive cancer risk genes. None of the tumors had a second mutation within the same gene. P/LP, pathogenic or likely pathogenic.
risk pediatric cohort, 22% of participants had at least one P/LP germline autosomal dominant or recessive variant, similar to rates seen in other cohorts.2,20-22 Four major conclusions from our study emerged: (1) Germline variants are likely to be included in tumor-only sequencing reports, (2) some therapeutic recommendations may be made in reference to germline variants, (3) germline P/LP variants of clinical significance may be missed by tumor-only sequencing because of filtering on the basis of population frequency or masked by copy-number alterations; and (4) patients may be unnecessarily notified of a potential hereditary risk related to a finding that ultimately turns out not to be germline.

Within this pediatric cohort, more than half of the SNVs reported from tumor-only sequencing were actually germline, including 31% of tier 1 and 2 variants. Consistent with prior reports, we found that the distribution of VAFs of somatic and germline variants overlap and VAFs alone therefore cannot be
used to reliably distinguish the germline or somatic origin of individual variants.\(^{23}\) We also found that 24% of the iCat recommendations were made on the basis of variants later confirmed to be germline. Although the utility of targeting some germline variants remains unclear, there are, on the other hand, P/LP germline variants that have established therapeutic relevance: \(BRCA1\) and \(BRCA2\) variants and response to poly(ADP-ribose) polymerase (PARP) inhibitors, \(KIT\) and \(PDGFRB\) variants and tyrosine kinase inhibitors, and mismatch repair gene variants and immune checkpoint inhibitors.\(^{24-26}\) Therefore, accurately identifying variants as germline first and then determining whether these have therapeutic relevance remains essential to consider.

Identifying hereditary P/LP variants in cancer predisposition genes may additionally affect future cancer screening or family planning for the patient and their family members.

We found that tumor-only sequencing was not sufficient to identify all the clinically significant germline variants because of filtering of variants present above predefined thresholds in population databases, as well as copy-number gains and losses within the tumor. Although strategies to recover known P/LP variants in key genes can be part of the analytic pipeline, it can be difficult to make such recovery strategies comprehensive. Ideally, a paired tumor-normal analysis will allow for the optimal identification and recognition of significant germline alterations.

**TABLE 2.** Treatment Recommendations on the Basis of Tumor-Only Analysis Include Germline Variants

| Variant | Germline Classification | Comments Within Report | Tier |
|---------|-------------------------|------------------------|------|
| \(CHEK2\) c.1100delC (p.T367Mfs*15) | P | Caveat in report regarding gene association. Flagged as possible germline. | 5 |
| \(FAM175A\) c.1106dupG (p.S370Ifs*2) | P | Flagged as possible germline | 4 |
| \(SDHA\) c.91C>T (p.R31*) | P | None | 3 |
| \(SMARCA4\) c.948delT (p.A317Pfs*9) | P | Flagged as possible germline | 2 |
| \(TP53\) c.742C>T (p.R248W) | P | Flagged as possible germline | 2 |
| \(TP53\) c.743G>A (p.R248Q) | P | Flagged as possible germline | 2 |
| \(TP53\) c.392A>T (p.N131I) | LP | Flagged as possible germline | 2 |
| \(AKT1\) c.1112C>A (p.T371K) | VUS | Caveat in report regarding VUS | 2 |
| \(NBN\) c.456G>A (p.M152I) | VUS | Caveat in report regarding VUS | 4 |
| \(TP53\) c.949C>A (p.Q317K) | VUS | Caveat in report regarding VUS. Flagged as possible germline. | 5 |
| \(TSC2\) c.2656G>C (p.V886L) | VUS | Caveat in report regarding VUS | 2 |

**NOTE.** Eleven treatment recommendations were based on single-nucleotide variants that were later confirmed to be germline. Most (64%) were flagged as possibly germline during clinical review. The therapeutic recommendations were classified within tiers that indicate the level of evidence supporting recommendations (Tier 2: clinical evidence demonstrating a benefit for targeted therapy in patients with a different tumor and a variant in the same gene. Tier 3: preclinical evidence demonstrating a benefit for targeted therapy in models of the same tumor type. Tier 4: preclinical evidence demonstrating a benefit for targeted therapy in models of a different tumor type. Tier 5: expert panel feels there is insufficient information to qualify for treatment recommendation).

Abbreviations: LP, likely pathogenic; P, pathogenic; VUS, variant of uncertain significance.
because of the risk of masking important germline variants using a subtraction method.26

Our data support that paired tumor-normal sequencing would be best performed as an integrated analysis within the NGS pipeline. This approach would drastically decrease the workload for the molecular pathologist, shorten the time to clinical report, and greatly simplify the interpretation of results for the receiving provider or patient, which are time-intensive and potentially prohibitive.27,28 It is important to acknowledge that many barriers exist to performing paired tumor-normal analysis including challenges in collecting tumor and blood samples simultaneously, limited access to molecular pathologists experienced in curating both somatic and germline cancer gene variants, and a potentially longer, more complicated consent process.

Our study had several limitations. First, our study was restricted to patients with pediatric solid tumors, well known to have lower somatic mutation rates than other cancers. Data also suggest that germline alterations may contribute more to the transformation process of early-onset cancers.29 In addition, our study was limited to the analysis of variants within 147 of 447 genes included in the tumor sequencing. It is possible that the proportion of germline variants would be different if every gene were examined within the larger gene list. We also observed that there were very few cases within our cohort of tumor samples with elevated tumor mutational burden and, therefore, were unable to assess the impact that tumor-only sequencing may have had on these estimations, but we acknowledge that this remains another important consideration.30 Finally, it is important to note that sequencing assays and associated analytic pipelines may be distinct, such that the results shown here may not overlap perfectly with other precision medicine programs that rely on different analytic tools for their tumor-only sequencing. Despite these limitations, the benefits of integrated germline sequencing, when feasible, are expected to be broadly applicable.

In summary, we demonstrate several potential gaps in the interpretation of tumor-only sequencing data in pediatric high-risk tumors and suggest that paired tumor-normal sequencing and analysis may offer substantial benefits for cancer precision medicine programs.

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REFERENCES

1. Harris MH, DuBois SG, Glade Bender JL, et al: Multicenter feasibility study of tumor molecular profiling to inform therapeutic decisions in advanced pediatric solid tumors: The individualized cancer therapy (iCat) study. JAMA Oncol 2:608-615, 2016

2. Parsons DW, Roy A, Yang Y, et al: Diagnostic yield of clinical tumor and germline whole-exome sequencing for children with solid tumors. JAMA Oncol 2:616-624, 2016

3. Cheng DT, Mitchell TN, Zehir A, et al: Memorial Sloan Kettering-integrated mutation profiling of actionable cancer targets (MSK-IMPACT): A hybridization capture-based next-generation sequencing clinical assay for solid tumor molecular oncology. J Mol Diagn 17:251-264, 2015

4. Cheng DT, Prasad M, Chekulay W, et al: Comprehensive detection of germline variants by MSK-IMPACT, a clinical diagnostic platform for solid tumor molecular oncology and concurrent cancer predisposition testing. BMC Med Genomics 10:33, 2017

5. Rusch M, Nakland W, Shurtleff S, et al: Clinical cancer genomic profiling by three-platform sequencing of whole genome, whole exome and transcriptome. Nat Commun 9:3962, 2018

6. Beaubier N, Bontrager M, Huether R, et al: Integrated genomic profiling expands clinical options for patients with cancer. Nat Biotechnol 37:1351-1360, 2019

7. Merker JD, DeVereaux K, Iafraite K, et al: Proficiency testing of standard samples shows very high interlaboratory agreement for clinical next-generation sequencing-based oncology assays. Arch Pathol Lab Med 143:463-471, 2019

8. Sukhraj MA, Misra N, Thomas M, et al: Somatic tumor variant filtration strategies to optimize tumor-only molecular profiling using targeted next-generation sequencing panels. J Mol Diagn 21:261-273, 2019

9. McNulty SN, Parikh BA, Duncavage EJ, et al: Optimization of population frequency cutoffs for filtering common germline polymorphisms from tumor-only next-generation sequencing data. J Mol Diagn 21:903-912, 2019

10. Zehir A, Benayed R, Shah RH, et al: Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. Nat Med 23:703-713, 2017

11. Jones S, Anagnostou V, Lyle K, et al: Personalized genomic analyses for cancer mutation discovery and interpretation. Sci Transl Med 7:283ra53, 2015

12. Wagle N, Berger MF, Davis MJ, et al: High-throughput detection of actionable genomic alterations in clinical tumor samples by targeted, massively parallel sequencing. Cancer Discov 2:82-93, 2012

13. Abo RP, Ducar M, Garcia EP, et al: BreaKmer: Detection of structural variation in targeted massively parallel sequencing data using kmers. Nucleic Acids Res 43:e19, 2015

14. Garcia EP, Minkovsky A, Jia Y, et al: Validation of OncoPanel: A targeted next-generation sequencing assay for the detection of somatic variants in cancer. Arch Pathol Lab Med 141:751-758, 2017

15. Sholl LM, Do K, Shividashi P, et al: Institutional implementation of clinical tumor profiling on an unselected cancer population. JCI Insight 1:e87062, 2016

16. Nowak JA, Yurgelen MB, Bruce JL, et al: Detection of mismatch repair deficiency and microsatellite instability in colorectal adenocarcinoma by targeted next-generation sequencing. J Mol Diagn 19:84-91, 2017

17. 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 and College of American Pathologists. J Mol Diagn 19:4-23, 2017

18. Manning DK, Garcia EP, Davineni PK, et al: Adaptation and validation of a pan-cancer somatic next generation sequencing assay for detection of germline hereditary cancer predisposition variants. J Mol Diagn 20:895-1039, 2018

19. 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 17:405-424, 2015

20. Fiala EM, Jayakumaran G, Mauguen A, et al: Prospective pan-cancer germline testing using MSK-IMPACT informs clinical translation in 751 patients with pediatric solid tumors. Nat Cancer 2:357-365, 2021

21. MacFarland SP, Zelley K, Surrey LF, et al: Pediatric somatic tumor sequencing identifies underlying cancer predisposition. JCO Precis Oncol 10.1200/po.19.00062

22. Zhang J, Walsh MF, Wu G, et al: Germline mutations in predisposition genes in pediatric cancer. N Engl J Med 373:2336-2346, 2015

23. Montgomery ND, Selitsky SR, Patel NM, et al: Identification of germline variants in tumor genomic sequencing analysis. J Mol Diagn 20:123-125, 2018

24. Mohyuddin GR, Aziz M, Britt A, et al: Similar response rates and survival with PARP inhibitors for patients with solid tumors harboring somatic versus germline BRCA mutations: A meta-analysis and systematic review. BMC Cancer 20:507, 2020

25. Faraco I, Graziano G: Role of BRCA mutations in cancer treatment with poly(ADP-ribose) polymerase (PARP) inhibitors. Cancers (Basel) 10:487, 2018

26. Thavaneswaran S, Rath E, Tucker K, et al: Therapeutic implications of germline genetic findings in cancer. Nat Rev Clin Oncol 16:386-396, 2019

27. Hirsch S, Gieldon L, Sutter C, et al: Germline testing for homologous recombination repair genes-opportunities and challenges. Genes Chromosomes Cancer 60:332-343, 2021

28. RaviChandar V, Shamer Z, Kemel Y, et al: Toward automation of germline variant curation in clinical cancer genetics. Genet Med 21:2116-2125, 2019

29. Qing T, Mohsen H, Marczyk M, et al: Germline variant burden in cancer genes correlates with age at diagnosis and somatic mutation burden. Nat Commun 11:2438, 2020

30. Parikh K, Huether R, White K, et al: Tumor mutational burden from tumor-only sequencing compared with germline subtraction from paired tumor and normal specimens. JAMA Netw Open 3:e200202, 2020
**FIG A1.** Pairwise comparison of germline VAFs in tumor versus normal tissue. Pairwise comparison of the AF of germline variants identified from tumor-only sequencing (tumor tissue) to the AF from germline sequencing (normal tissue). Each variant is color-coded to indicate the copy-number status (amplification, gain, single copy deletion, or neutral) of the corresponding gene. Copy-number gains and loss could not explain many germline VAFs outside of 40%-60% in tumor tissue. AF, allelic fraction; CN, copy number; VAF, variant allele fraction.
| Gene (Ensembl Reference Transcript, Variant) | Cancer Risk Classification | Tumor Diagnosis | Present on Tumor-Only Sequencing Report | Second Mutation in Tumor | Gene Previously Associated With Cancer Type |
|---------------------------------------------|---------------------------|----------------|---------------------------------------|--------------------------|---------------------------------------------|
| **APC** (ENST00000457016), c.3920T>A (p.I1307K) | AD | Osteosarcoma | No | No | No |
| **APC** (ENST00000457016), c.3920T>A (p.I1307K) | AD | Wilms tumor | Yes | No | No |
| **BLM** (ENST00000355112), c.1933C>T (p.Q645*) | AR | Ewing sarcoma | Yes | No | No |
| **BRCA1** (ENST00000357654), CNV intron9/exon 10 partial deletion | AD | Neuroblastoma | No | No | No |
| **CHEK2** (ENST00000404276), c.1100delC (p.C1197*) | AD | Wilms tumor | Yes | No | No |
| **DICER1** (ENST00000393063), c.3591C>A (p.C1197*) | AD | CNS sarcoma | Yes | **DICER1** c.5125G>A (p.D1709N) | Yes |
| **DICER1** (ENST00000393063), c.1525C>T (p.R509*) | AD | Pleuropulmonary blastoma | Yes | **DICER1** c.5113G>A (p.E1705K) | Yes |
| **DICER1** (ENST00000393063), c.2781C>T (p.Y927*) | AD | Uterine embryonal rhabdomyosarcoma, botryoid type | Yes | **DICER1** c.5125G>A (p.D1709N) | Yes |
| **DICER1** (ENST00000393063), c.904-1G>C | AD | Sertoli-Leydig cell tumor of the ovary | Yes | **DICER1** c.5439G>T (p.E1813D) | Yes |
| **DOCK8** (ENST00000453981), c.700delA (p.R234Gfs*40) | AR | Alveolar rhabdomyosarcoma | Yes | No | No |
| **ELANE** (ENST00000263621), whole gene deletion | AD | CIC rearranged sarcoma | Yes | No | No |
| **ERCC3** (ENST00000285398), c.325C>T (p.R109*) | AR | Mesenchymal chondrosarcoma | No | No | No |
| **FAM175A** (ENST00000321945), c.1106dupG (p.T75del) | AD | Osteosarcoma | Yes | Deletion of **FAM175A** | No |
| **FANCA** (ENST00000389301), CNV exons 11-29 deletion | AR | Hepatoblastoma | Yes | No | No |
| **GBA** (ENST00000368373), c.1226A>G (p.N409S) | AR | Mesenchymal chondrosarcoma | No | No | No |
| **GBA** (ENST00000368373), c.1226A>G (p.N409S) | AR | CIC rearranged sarcoma | No | No | No |
| **GBA** (ENST00000368373), c.222_224delTAC (p.T75del) | AR | Osteosarcoma | Yes | No | No |
| **MITF** (ENST00000352241), c.1255G>A (p.E419K) | AD | Ewing sarcoma | No | No | No |
| **MUTYH** (ENST00000372098), c.1178G>A (p.G393D) | AR | Spindle cell neoplasm (infantile myofibroma) | Yes | No | No |
| **MUTYH** (ENST00000372098), c.1178G>A (p.G393D) | AR | Alveolar rhabdomyosarcoma | Yes | No | No |

(Continued on following page)
| Gene (Ensembl Reference Transcript), Variant | Cancer Risk Classification | Tumor Diagnosis | Present on Tumor-Only Sequencing Report | Second Mutation in Tumor | Gene Previously Associated With Cancer Type |
|---------------------------------------------|---------------------------|----------------|----------------------------------------|--------------------------|-----------------------------------------|
| MUTYH (ENST00000372098), c.303C>A (p.Y101*) | AR | Hepatocellular carcinoma | Yes | No | NA |
| RB1 (ENST00000267163), c.1216-3A>G | AD | Osteosarcoma | Yes | No | Yes |
| SBDS (ENST00000246868), c.258+2T>C | AR | Osteosarcoma | No | No | No |
| SDHA (ENST00000264932), c.91C>T (p.R31*) | AD | GIST | Yes | Deletion of SDHA | Yes |
| SERPINA1 (ENST00000440909), c.1096G>A (p.E366K) | AR | Osteosarcoma | No | No | No |
| SERPINA1 (ENST00000440909), c.1096G>A (p.E366K) | AR | Osteosarcoma | No | No | No |
| SERPINA1 (ENST00000440909), c.1096G>A (p.E366K) | AR | Osteosarcoma | No | No | No |
| SERPINA1 (ENST00000440909), c.187C>T (p.R63C) | AR | Ewing sarcoma | Yes | No | No |
| SERPINA1 (ENST00000440909), c.739C>T (p.R247C) | AR | Ganglioneuroblastoma | No | No | No |
| SMARCA4 (ENST00000344626), c.548delT (p.A317Pfs*9) | AD | Malignant neoplasm with rhabdoid-like morphology | Yes | CN-LOH | Yes |
| TP53 (ENST00000269305), c.392A>T (p.N131I) | AD | Chondrosarcoma | Yes | No | Yes |
| TP53 (ENST00000269305), c.742C>T (p.R248W) | AD | Osteosarcoma | Yes | Deletion of TP53 within loss of 17p | Yes |
| TP53 (ENST00000269305), c.743G>A (p.R248Q) | AD | Osteosarcoma | Yes | CN-LOH | Yes |
| UROD (ENST00000246337), c.239C>G (p.A80G) | AD | Neuroblastoma | Yes | No | No |
| WRN (ENST00000298139), c.2194C>T (p.R732*) | AR | Sertoli-Leydig cell tumor of the ovary | Yes | No | No |
| XPA (ENST00000375128), c.555G>C (p.Q185H) | AR | Osteosarcoma | Yes | No | No |

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; CN-LOH, copy neutral loss of heterozygosity; CNS, central nervous system; CNV, copy-number variant; GIST, gastrointestinal stromal tumor.