Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.
eMethods. Detailed Methods

All patients in this study were referred for germline testing to Invitae (San Francisco, CA). The specific genes tested varied at clinician discretion, although multi-gene panels were ordered for most patients in this study (Table 1). Genes with low penetrance (i.e., conferring relatively low cancer risks) and specific low penetrance variants were excluded from this study, even if clinically reported. These included \textit{APC} I1307K, \textit{CHEK2} I157T, \textit{HOXB13} G84E, as well as all \textit{MUTYH} heterozygotes and \textit{CFTR} variants, among others. (Note that some of these excluded findings may have been clinically actionable for family counseling or clinical trial eligibility.) We also excluded germline mosaic variants that were likely a result of clonal hematopoiesis, although follow-up testing (e.g., of fibroblasts or of family members) can be required to fully resolve these cases, and such results were not available to this study.

Clinical information provided on germline test requisition forms (TRFs) was used in this study, including patient demographic characteristics, cancer type(s), age at diagnosis, and personal history. TRF information and germline test results were queried for all patients receiving germline testing for hereditary cancer predisposition genes between January 2015 and January 2020. These records were manually filtered to include only those patients who (a) had a current diagnosis or personal history of cancer, and (b) had previously received tumor DNA sequencing according to the TRF. Other types of tumor tests (e.g., immunohistochemistry, microsatellite instability, or tumor mutation burden) were not considered in this study.

Ordering clinicians often summarized tumor sequencing results on TRFs, listing the mutations identified. Detailed lab reports for these tumor tests were attached to the germline TRFs for a subset of patients. These lab reports were retrieved and reviewed as needed to clarify information in the TRF. Lab reports were also reviewed whenever they were available for patients with an apparent discordance between the tumor sequencing and germline testing (i.e. those patients with variants listed in Table 2 and eTable 2). Additional lab reports were retrieved as a quality control check (see eResults). Patients were considered ineligible for our study whenever TRF information was not clear and tumor test reports were not available to clarify.

This work was conducted according to the STROBE recommendations for cohort studies (https://www.equator-network.org/reporting-guidelines/strobe/).
eResults. Detailed Results

In total, our database queries identified 2,512 potentially eligible patients, of whom 2,023 were determined to be eligible following manual review. A de-identified dataset was constructed for these 2023 patients and was used in this research study.

Tumor testing was performed by a variety of laboratories, most commonly (approximately 50% of patients) by Foundation Medicine, Inc. (Cambridge, MA). The vast majority of tumor tests involved sequencing of DNA from formalin-fixed paraffin-embedded (FFPE) tumor tissue, either biopsy or resection specimens, and most of these tests used hybridization based sequencing library preparation methods. A small fraction (less than 5%) of tests were liquid biopsies of non-hematological cancers using peripheral blood. An even smaller fraction (2%) were paired tumor-normal tests (FFPE and blood), although in most of these cases the normal tissue was sequenced primarily to subtract germline variants from the tumor variants in order to best measure somatic mutation. In such tests, pathogenic germline variants may be uncovered in the normal tissue, but if so, these results are not considered part of the validated CLIA test result, and follow-up CLIA germline testing is indicated. Roughly two-thirds of the 2% of patients with paired tests had pathogenic germline variants initially uncovered in this manner. This small group of patients did not have a significant effect on our overall germline positive rate (30.5% of 2,023 patients).

The descriptive percentages mentioned above are approximate because detailed tumor test information was not provided for many patients even when summarized test results were provided. Of note, Foundation Medicine test reports, and those of many other tumor testing laboratories in this study, provide limited, if any, technical information about whether the variants uncovered are potentially of germline origin (e.g., variant allele fractions and specimen tumor cellularity were rarely described, and recommendations for genetics referral or follow-up germline testing were rarely made).

In total, detailed tumor test lab reports were available, retrieved and reviewed for 9.5% of our 2,023 patients. Corresponding TRF information was compared to provide an estimate of the overall accuracy of TRF summaries. Other than minor typographic errors and oversimplifications (e.g., “BRCA” instead of “BRCA1”), we observed that certain genes were systematically omitted by clinicians on TRFs. In particular, genes for which most mutations are somatic and for which rarely have germline relevance (e.g., KRAS or BRAF) were rarely included in TRF summaries even when reported by tumor sequencing. Importantly however, we similarly saw that TP53 was under-reported in TRFs for approximately 60% of patients with mutations uncovered by tumor sequencing, and PTEN was under-reported in approximately 40%.
Patients in our study were referred by more than 200 different clinics, including both academic hospitals and community oncology or surgery practices. The stated reasons for ordering germline testing varied (see Methods) but were often not provided explicitly on TRFs, making their distribution difficult to quantify. Because of the variable degree of detail provided on TRFs, we were also unable to systematically evaluate whether patients would have met germline testing criteria based on detailed personal and family history factors. However, adequate information was available to evaluate whether patients would have met ESMO (European Society for Medical Oncology) and NCCN (National Comprehensive Cancer Network) germline follow-up testing criteria based on their tumor sequencing results and other applicable factors (e.g., age and cancer type).
eTable 1. Patients With Multiple Sequential Primary Cancers (N=69).a

| Age at 1st cancer diagnosis | Sex | Gene(s) with PGVs | Order of primary cancers | Screening or risk reduction recommended for subsequent cancer(s) |
|-----------------------------|-----|-------------------|--------------------------|---------------------------------------------------------------|
| 50-59                       | M   | ATM               | esophageal, gastric      |                                                               |
| 0-19                        | F   | ATM               | Other, other             |                                                               |
| 50-59                       | M   | ATM               | PRO, bladder, lung, PANC | Yesb                                                          |
| 70-79                       | M   | ATM               | PRO, CRC                 | Limited evidence                                              |
| 60-69                       | M   | ATM               | PRO, other               |                                                               |
| 50-59                       | F   | ATM, PALB2        | CRC, Amp/BD              | Yesb                                                          |
| 30-39                       | F   | BRCA1             | BR, CNS                  |                                                               |
| 50-59                       | F   | BRCA1             | BR, CRC                  |                                                               |
| 40-49                       | F   | BRCA1             | BR, lung                 |                                                               |
| 40-49                       | F   | BRCA1             | BR, OV                   | Yes                                                           |
| 70-79                       | F   | BRCA1             | BR, OV                   | Yes                                                           |
| 30-39                       | F   | BRCA1             | BR, ENDO/uterine         | Limited Evidence                                              |
| 30-39                       | F   | BRCA1             | BR, ENDO/uterine         | Limited Evidence                                              |
| 40-49                       | F   | BRCA1             | Cervix, thyroid, OV      | Yes                                                           |
| 60-69                       | F   | BRCA1             | Heme, BR, lung           | Yes                                                           |
| 70-79                       | M   | BRCA1             | Heme, CRC                |                                                               |
| 60-69                       | M   | BRCA1             | PRO, lung                |                                                               |
| 0-19                        | M   | BRCA1, RB1        | Other, GIST              |                                                               |
| 30-39                       | F   | BRCA2             | BR, CNS                  |                                                               |
| 50-59                       | F   | BRCA2             | BR, gastric              |                                                               |
| 60-69                       | F   | BRCA2             | BR, lung                 |                                                               |
| 30-39                       | F   | BRCA2             | BR, lung                 |                                                               |
| 40-49                       | F   | BRCA2             | BR, OV                   | Yes                                                           |
| 60-69                       | F   | BRCA2             | BR, OV                   | Yes                                                           |
| Age at 1st cancer diagnosis | Sex | Gene(s) with PGVs | Order of primary cancers | Screening or risk reduction recommended for subsequent cancer(s) |
|-----------------------------|-----|-------------------|--------------------------|---------------------------------------------------------------|
| 50-59                       | F   | BRCA2             | BR, OV                   | Yes                                                           |
| 50-59                       | F   | BRCA2             | BR, PANC                 | Yes                                                           |
| 60-69                       | F   | BRCA2             | BR, PANC                 | Yes                                                           |
| 30-39                       | F   | BRCA2             | BR, PANC                 | Yes                                                           |
| 60-69                       | F   | BRCA2             | BR, PANC                 | Yes                                                           |
| Unk                         | F   | BRCA2             | CRC, thyroid             |                                                               |
| 50-59                       | F   | BRCA2             | CRC, ENDO/uterine        | Limited Evidence                                              |
| 60-69                       | M   | BRCA2             | Gastric, CRC             |                                                               |
| 20-29                       | F   | BRCA2             | Heme, BR, esophageal     | Yes                                                           |
| 50-59                       | M   | BRCA2             | Heme, PANC               | Yes                                                           |
| 40-49                       | M   | BRCA2             | Melanoma, PANC           | Yes                                                           |
| 50-59                       | M   | BRCA2             | Melanoma, PRO, skin, bladder | Yes                                           |
| 30-39                       | M   | BRCA2             | Other, other             |                                                               |
| 0-19                        | M   | BRCA2             | Other, other             |                                                               |
| 60-69                       | M   | BRCA2             | Other, PRO               | Yes                                                           |
| 70-79                       | M   | BRCA2             | PRO, cholangiocarcinoma  | Yes\(^b\)                                                     |
| 60-69                       | M   | BRCA2             | PRO, cholangiocarcinoma  | Yes\(^b\)                                                     |
| 70-79                       | M   | BRCA2             | PRO, CUP                 |                                                               |
| 50-59                       | M   | BRCA2             | PRO, lung                |                                                               |
| 60-69                       | M   | BRCA2             | PRO, lung                |                                                               |
| 40-49                       | F   | BRCA2             | Thyroid, BR, PANC        | Yes                                                           |
| 60-69                       | F   | BRCA2, CHEK2      | BR, OV                   | Yes                                                           |
| 60-69                       | M   | BRCA2, NBN        | Urothelial, PRO, renal   | Yes                                                           |
| 70-79                       | M   | BRIP1              | Lung, CNS                |                                                               |
| 60-69                       | M   | CDH1              | PRO, gastric             | Yes                                                           |
| Age at 1st cancer diagnosis | Sex | Gene(s) with PGVs | Order of primary cancers | Screening or risk reduction recommended for subsequent cancer(s) |
|-----------------------------|-----|------------------|--------------------------|---------------------------------------------------------------|
| Unk                         | F   | CDKN2A           | Leiomyosarcoma, heme, BR |                                                               |
| 40-49                       | F   | CHEK2            | BR, CNS                  |                                                               |
| 40-49                       | F   | CHEK2            | BR, thyroid              | Limited evidence                                              |
| 20-29                       | M   | CHEK2            | CNS, lung                |                                                               |
| Unk                         | F   | CHEK2            | Heme, CRC                | Yes                                                           |
| 40-49                       | F   | CHEK2            | Other, lung              |                                                               |
| 60-69                       | F   | CHEK2            | Renal, CNS, lung         |                                                               |
| 30-39                       | M   | MEN1             | Other, other             | Yes                                                           |
| 50-59                       | M   | MLH1             | CRC, skin                | Limited evidence                                              |
| 40-49                       | M   | MLH1, TP53       | CRC, other, gastric      | Yes                                                           |
| 40-49                       | F   | MSH2             | ENDO/uterine, BR         | Limited evidence                                              |
| 40-49                       | F   | MSH2             | OV, other                |                                                               |
| 60-69                       | F   | PALB2            | BR, cholangiocarcinoma   | Yes                                                            |
| 70-79                       | F   | PALB2            | BR, esophageal           |                                                               |
| 60-69                       | M   | PMS2             | PRO, urothelial, melanoma|                                                               |
| Unk                         | F   | PTEN             | BR, ENDO/uterine         | Yes                                                           |
| 50-59                       | F   | TP53             | BR, ENDO/uterine         | Yes                                                           |
| 40-49                       | F   | TP53             | BR, leiomyosarcoma        | Yes                                                           |
| 20-29                       | F   | TP53             | Other, melanoma, CNS, CRC| Yes                                                           |
| 30-39                       | F   | VHL              | Skin, ENDO/uterine, OV   |                                                               |

*Patients listed had multiple primary cancers in a known time sequence, diagnosed at least one year apart, and harbored one or more pathogenic germline variants (PGVs) in the gene(s) listed. Entries are sorted by gene. 18 additional patients with PGVs (not shown) had multiple primary cancers, but either these cancers were synchronous, or the order in which these cancers were diagnosed was not clear.

*Screening for pancreatic cancer recommended, depending on family history. Such screening would likely detect ampullary or bile duct cancers, or cholangiocarcinomas.

*Expert opinion would recommend a dermatology referral for Lynch syndrome patients, although the NCCN guidelines do not currently make such a recommendation.
Abbreviations: Amp/BD, ampullary or bile duct; BR, breast; CNS, central nervous system; CRC, colorectal; CUP, cancer of uncertain primary; ENDO, endometrial; GIST, gastrointestinal stromal tumor; heme, hematological cancer; NCCN, National Comprehensive Cancer Network; OV, ovarian; PANC, pancreatic; PGVs, pathogenic germline variants; PRO, prostate; Unk, unknown.
### eTable 2. Germline Findings Not Reported by Tumor Tests (N=50).a

| Gene  | Variant | Protein impact | Tumor test(s) | Reason for difference |
|-------|---------|----------------|---------------|-----------------------|
| ATM   | c.103C>T | p.Arg35*       | Tissue        | Gene not on tumor test |
| BRCA2 | C.8967_8973del TTGGCGT | p.Trp2990Hisfs*9 | Tissue | Gene not on tumor test |
| PMS2  | c.943C>T | p.Arg315*      | Tissue        | Gene not on tumor test |
| PMS2  | c.2174+1G>A | Splice donor  | Tissue        | Gene not on tumor test |
| CHEK2 | c.909-2A>G | Splice acceptor | Tissue        | Gene/Region not on tumor test |
| MLH1  | c.1975C>T | p.Arg659*      | Tissue        | Gene/Region not on tumor test |
| BRIP1 | c.2992_2995delAAGA | p.Lys998Glu7fs*9 | Liquid biopsy | Gene not on tumor test |
| BRIP1 | c.2392C>T | p.Arg798*      | Liquid biopsy | Gene not on tumor test |
| CHEK2 | c.1100delC | p.Thr367Metfs*15 | Liquid biopsy | Gene not on tumor test |
| SDHAF2| c.165G>A | p.Trp55*       | Liquid biopsy | Gene not on tumor test |
| NBN   | c.2099del | p.Pro7000Hisfs*9 | Tissue        | Gene not on tumor test |
| PMS2  | c.137G>T | p.Ser46Ile     | Tissue        | Gene not on tumor test |
| RAD51C| c.97C>T | p.Gln33*       | Tissue        | Gene not on tumor test |
| PMS2  | c.2113G>A | p.Glu705Lys    | Tissue        | Known technical limitation (pseudogene associated region) |
| PMS2  | c.2175-1G>C | Splice acceptor | Tissue        | Known technical limitation (pseudogene associated region) |
| PMS2  | c.1855del | p.ASp619Thrfs*4 | Tissue        | Known technical limitation (pseudogene associated region) |
| ATM   | c.8851-?_3591+?del | Deletion (Exons 62-63) | Tissue | Likely technical limitation (small CNV deletion) |
| CDH1  | c.2165-?_2042+?del | Deletion (Exons 14-16) | Tissue | Likely technical limitation (small CNV deletion) |
| CHEK2 | c.909-?_1095+?del | Deletion (Exons 9-10) | Tissue | Likely technical limitation (small CNV deletion) |
| Gene   | Variant         | Protein impact                      | Tumor test(s)   | Reason for difference                      |
|--------|-----------------|-------------------------------------|-----------------|--------------------------------------------|
| CHEK2  | c.909-?_1095+?del | Deletion (Exons 9-10)               | Tissue          | Likely technical limitation (small CNV deletion) |
| CHEK2  | c.320-?_592+?del | Deletion (Exons 3-4)                | Tissue          | Likely technical limitation (small CNV deletion) |
| CDKN2A | c.9_32dup       | p.Ala4_Pro11dup                     | Tissue          | Likely technical limitation (complex variant) |
| CDKN2A | c.9_32dup       | p.Ala4_Pro11dup                     | Tissue          | Likely technical limitation (complex variant) |
| TP53   | c.742C>T        | p.Arg248Trp                         | Tissue          | Likely technical limitation (allele drop out) |
| CDKN2A | c.458-105A>G    | Intrinsic                           | Liquid biopsy   | Likely technical limitation (intrinsic variant) |
| BRCA1  | c.1175_1214del  | p.Leu392Glnfs*5                     | Liquid biopsy   | Likely technical limitation (large indel)   |
| ATM    | c.357dupA       | p.Leu120Thrfs*14                    | Tissue          | Possible technical limitation               |
| NBN    | c.657_661delACAAA | p.Lys219Asnfs*16                   | Tissue          | Variant interpretation difference           |
| NF1    | c.2540T>G       | p.Leu847Arg                         | Tissue          | Variant interpretation difference           |
| SDHB   | c.649C>T        | p.Arg217Cys                         | Tissue          | Variant interpretation difference           |
| CHEK2  | c.190G>A        | p.Glu64Lys                          | Tissue          | Likely variant interpretation difference     |
| CHEK2  | c.190G>A        | p.Glu64Lys                          | Tissue          | Likely variant interpretation difference     |
| CHEK2  | c.190G>A        | p.Glu64Lys                          | Tissue          | Likely variant interpretation difference     |
| CHEK2  | c.536A>G        | p.Tyr179Cys                         | Tissue          | Likely variant interpretation difference     |
| CHEK2  | c.433C>T        | p.Arg145Trp                         | Tissue          | Likely variant interpretation difference     |
| NBN    | c.1124+2T>G     | Splice donor                        | Tissue          | Likely variant interpretation difference     |
| TP53   | c.742C>T        | p.Arg248Trp                         | Tissue          | Likely variant interpretation difference     |
| TP53   | c.817C>T        | p.Arg273Cys                         | Tissue          | Likely variant interpretation difference     |
| Gene   | Variant    | Protein impact       | Tumor test(s)   | Reason for difference                                      |
|--------|------------|----------------------|-----------------|------------------------------------------------------------|
| ATM    | c.2228C>G  | p.Ser743*            | Tissue          | Unknown (gene was on tumor test)                           |
| BRCA1  | c.5266dup  | p.Gln1756Profs*74    | Tissue          | Unknown (gene was on tumor test)                           |
| CHEK2  | c.1100delC | p.Thr367Metfs*15     | Tissue and liquid biopsy | Unknown (gene was on tumor test)                           |
| CHEK2  | c.1100delC | p.Thr367Metfs*15     | Tissue          | Unknown (gene was on tumor test)                           |
| PALB2  | c.697del   | p.Val233Leufs*5      | Tissue          | Unknown (gene was on tumor test)                           |
| PTEN   | c.802-1G>A | Splice acceptor      | Tissue          | Unknown (gene was on tumor test)                           |
| RAD51C | c.525dupC  | p.Cys176Leufs*27     | Tissue          | Unknown (gene was on tumor test)                           |
| BRCA2  | c.5828del  | p.Ser1943Leufs*20    | Tissue          | Unknown                                                    |
| CHEK2  | c.1100delC | p.Thr367Metfs*15     | Tissue          | Unknown                                                    |
| CHEK2  | c.1254del  | p.Phe418Leufs*19     | Tissue          | Unknown                                                    |
| MSH2   | c.1759+1G>T| Splice donor         | Tissue          | Unknown                                                    |
| RAD51C | c.224dupA  | p.Tyr75*             | Tissue          | Unknown                                                    |

*Variants listed were uncovered by germline testing and were either not reported by tumor sequencing or were reported as being not clinically important. All of these were considered pathogenic or likely pathogenic by the germline test. Each row corresponds to one patient.

Abbreviations: CNV, copy number variant.