

Supplementary Online Content

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eReferences

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

eAppendix 1. Ascertainment, pre- and post test counseling, consent, and research testing.

Patients were ascertained by treating physicians according to a research protocol 12-245 in which eligibility included any patient with cancer treated at MSKCC who had previously consented to “Part A” of the study (tumor genotyping). Patients were enrolled without consideration to prior germline testing. (Indeed, prior genetic counseling was reported in 53 of the 205 cases who were found during the study to harbor mutations; in 30 cases the study confirmed the already known high penetrant mutation, and in 3 of these cases identified a second (incremental) variant in a low or moderate penetrant gene.)

Pre-test counseling involved discussion by the ordering physician-investigator and supplemented by an educational video. The video-assisted consent process included the elements covered in traditional pre-test counseling (concepts of inheritance, purpose of analysis, potential implications for family members, questions about genetic discrimination, test limitations). While uptake was not an element in the study, a pilot project of a subset of cases indicated that the level of uptake was in excess of 90% consenting to germline return of results as well as somatic (tumor) sequencing. The enrolling physicians were responsible for transmitting the test result to the patient once the results were available, with the recommendation to refer to post-test counseling. Patients who declined post-test counseling, according to the protocol, received the results from their physicians. Additional follow-up by clinical genetics staff was provided stating the availability of genetic counseling, and the potential implications of a germline result to the patient and family. For patients who came for genetic counseling after receiving the results from their physicians, the post-test counseling was not uniformly modified to account for a different pre-test counseling model, but was individualized depending on the extent of patient’s knowledge and understanding of the test results already disclosed to them by physicians.

Variants of uncertain significance (VUS) were not communicated in this study. However, the clinical laboratory providing this testing (called IMPACT) headed by Board-certified lab directors, reviewed VUS on a regular basis. Any variants initially classified as a VUS that were re-classified to likely pathogenic or pathogenic variants were communicated to the ordering physicians and/or Clinical Genetics Service and patients via amended clinical reports.

All of the germline- and tumor- sequencing- performed in this study was completed as part of a research study. There was no billing to patient for genetic testing, or pre test counseling. Individuals with positive test results coming for post test counseling were billed for post test counseling and consultation as appropriate and family members invited for follow up testing and counseling as per standard guidelines. Insurance or third party coverage for testing was not requested as it would not have been included in coverage for many cases and would have served to negatively impact and potentially bias ascertainment.

eAppendix 2. Methods for variant calling.

Germline variants from the .bam file of the non-tumor DNA sequence with mapping and base quality scores of ≥ 20 were called using MuTect¹ and GATK Haplotypecaller² using 25% variant frequency and 20X coverage thresholds. All variants with $< 1\%$ population frequency in the Exome Aggregation Consortium (ExAC) database were interpreted. Copy number variations were assessed using an in-house developed pipeline and were confirmed with an additional laboratory tests³.

eAppendix 3. Definitions of high, moderate, low penetrance variants, variants associated with recessive syndromes, and variants of uncertain clinical actionability.

Mutations were classified by penetrant status utilizing known disease risks and current modeling⁴⁻¹⁴. *BAP1*, *BRCA1/2*, *CDH1*, *CDKN2A*, *FH*, *FLCN*, *MEN1*, *MLH1*, *MSH2*, *MSH6*, *PALB2*, *PMS2*, *SDHA/B*, and *VHL* were considered “high penetrant”, according to the consensus guidelines and known associations with high-penetrance hereditary cancer predisposition syndromes^{5-7,9,14-19}. While *PALB2* has been referred to as moderate penetrance in some series, the OR of 5.3 in the recent analysis by Easton et al places *PALB2* in the higher penetrant category, defined by OR >4.0¹⁴. Mutations in *ATM*, *BRIP1*, *CHEK2* (other than p.Ile157Thr), *MITF*, *NBN*, and *RAD51D* genes were considered “moderate penetrance,” defined as OR = 2-4. The role of *ATM*, *CHEK2*, and *BRIP1*, *RAD51D* as moderate penetrance genes associated with increased risk of breast or ovarian cancer, respectively, has been well established^{5,11-15}, while the evidence regarding specific cancer risks associated with mutations in *NBN* and *MITF* is still emerging. *NBN* is considered a moderate penetrance predisposition gene associated with increased breast cancer risk, based largely on data related to a protein-truncating variant, c.657del5, shown by meta-analysis of 10 studies to have an OR of 2.7^{14,15}, with a single recent case-control study showing an association with pancreatic cancer (OR: 3.80)⁸. It remains unclear if other mutations of *NBN* will also show these same levels of disease risk, but for the purposes of this analysis the classification of *NBN* as moderate penetrance by Easton et al¹⁴ was observed. While the evidence regarding the level of risk associated with *MITF* mutations in kidney cancer has not been conclusive⁷, recent studies support the originally described status of *MITF* as a moderate penetrance predisposition gene in melanoma (OR 3.3⁹; OR: 2.85¹⁰), with recommendations given for heightened surveillance. *BRIP1* is also characterized as an ovarian cancer risk gene by NCCN, with OR's in the range of 8.13-11.22^{11,12,16}. *APC* p.Ile1307Lys and heterozygous mutations in *MUTYH* were coded as “low penetrant” variants (OR >1 but generally 2 or lower).

RECQL4 was classified as of unknown risk as a heterozygous variant, although of proven association with recessive syndromes, while *FAM175A*, *BARD1* and *RAD50* mutations and *CHEK2* Ile157Thr were classified as pathogenic mutations of uncertain clinical actionability in the preventive setting, and/or unproven but of potential utility as therapeutic targets. During the course of the study an in frame insertion in the fumarate hydratase gene (*FH* c.1431_1433dupAAA p.Lys477dup; rs367543046, chr1:241661227 A/ATTT) was found to recur among 5 individuals, 1 with pancreatic (diagnosed age 56) and 4 with prostate cancer (diagnosed ages 59, 65, 65, 71), 4 of whom were of self-identified Ashkenazi ancestry. Because none of these cases fulfilled diagnostic criteria for or had a family history of Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC), for the purposes of the current analysis, this variant was classified as correlated with recessive FH deficiency, but of uncertain clinical actionability with respect to HLRCC, until further genetic epidemiologic evidence is available.

eAppendix 4. Decision rules for family history classification according to published criteria for genomic testing.

For patients meeting criteria for genomic testing as established by NCCN, ACMG, or syndrome-specific guidelines where NCCN or ACMG not available,¹⁵⁻²⁰ and/or based on characteristic morphology associated with the known syndrome), disease-specific panel testing (Breast, Ovarian, Breast Ovarian, Colorectal, Polyposis, Pancreatic, Renal, Paraganglioma panels) were deemed appropriate according to the following rules:

- 1) Patients diagnosed with the tumor type corresponding to the disease-specific panel (breast, ovarian, colon, pancreatic or renal cancer) when there was no known mutation in the family that would fully account for the observed history. If there was a known mutation in the family, then the patient offered targeted gene test only.
- 2) Patients diagnosed with the tumor type corresponding to the disease-specific panel (breast, ovarian, colon, pancreatic or renal cancer), meeting criteria for >1 predisposition syndrome known to be associated with the tumor type and included in one of the panels

When criteria for testing were met solely based on Ashkenazi ancestry (e.g. late onset isolated breast cancer and Ashkenazi ancestry), targeted gene testing was performed as indicated by guidelines.

Panels were not considered appropriate for patients with non-syndromic tumor types who met diagnostic criteria for syndrome testing based on the family history, in which case directed gene testing was offered for breast ovarian, Li-Fraumeni or other syndromes as indicated.

Cases meeting testing criteria for specific syndromes based on histology alone were offered testing based on tumor morphology (e.g. fumarate hydratase for hereditary leiomyomatosis renal cell cancer syndrome).

eAppendix 5. Decision rules for determination of penetrance levels, clinically actionable status, and incremental status for cases with two variants.

For classification of number of patients with variants in genes of various penetrances, if the case had 2 variants with different penetrance levels, then the case was classified according to the higher penetrant variant. In all analyses no cases were counted more than once. In the analysis of incremental findings not predicted by clinical guidelines, when accounting for cases with both an actionable incremental variant and one that was not actionable or incremental, then those cases were counted as having an actionable incremental finding. If a case had both a low penetrant founder mutation and a higher penetrance non-founder mutation that was incremental to phenotype based analysis, that case was scored as having the higher penetrant variant and was not subtracted from total of incremental findings minus founder mutations. For cases with two variants where one variant that was predicted by phenotype and was of higher penetrance than a second variant that was incremental, for the analysis of incremental findings by penetrance class, the lower penetrant incremental variant was scored and counted in this analysis. In cases where a second cancer was diagnosed after the first, but the genetic analysis was performed on the first cancer, then that case was considered as having a primary diagnosis of the tumor type at time of genetic analysis. In the series, 15 cases had more than one variant, including 11 high penetrant cases also with a moderate, low, or recessive mutation, 2 moderate penetrant cases also with a low penetrant mutation, and 2 low penetrant cases with another low penetrant or variant of uncertain actionability.

eAppendix 6. Supplemental statistical methods.

1. Adjustment of incremental rates

Adjustment for Case Mix

To estimate the proportion of incremental variant detection in an unselected population of cancer patients, observed tumor type-specific rates of pathogenic or likely pathogenic genetic variants (P/LPGV) were multiplied by cancer rates based on general population incidence²¹, and then multiplied by tumor type-specific rates of incremental variants observed or imputed from a separate ascertainment²², and summed to give expected rates of incremental findings given case mix adjustment for purposes of estimated adjusted rates given a different case mix (Discussion section).

Case mix adjustment for the cohort was performed by deriving proportion of cancers of type A, B, C, etc in the study cohort that would have been expected using distributions of expected 2016 cancer incidence by the North American Association of Central Cancer Registries (NAACCR) as compiled by the American Cancer Society Surveillance Research unit²¹, and then deriving the adjusted number of incremental P/LPGV in the cohort that would have occurred given the adjustment in case mix, while preserving the rate of incremental findings empirically observed for each cancer type. This was derived as:

N_A = number of cases of cancer type A in the cohort studied

P_A = proportion of cases of cancer type A in the cohort, i.e. = $N_A / 1040$ for the current series

P_S = proportion of cases of cancer type A in NAACCR/ACS yearly 2016 incidence

N_{ADJ} = adjusted number of cases of cancer type A using NAACCR/ACS rates in cohort studied,

$$= N_A P_S / P_A \text{ (i.e.} = P_S \times 1040 \text{ in the current series)}$$

V_A = number of P/LPGV for cancer type A in study cohort (observed)

I_A = incremental number of P/LPGV for cancer type A in cohort (observed)

IF_A = incremental fraction of P/LPGV for cancer type A in cohort (observed) = I_A / V_A

V_{ADJ} = adjusted number of variants in cohort expected given adjusted number of cases of cancer type A = $V_A P_A / N_A$

I_{ADJ} = adjusted number of incremental P/LPGV in the cohort for cancer type A = $V_{ADJ} IF_A$

For entire cohort for all cancer types:

$$\sum I_{\text{ADJ}} / \sum V_{\text{ADJ}} = \text{proportion of incremental P/LPGV in total cohort}$$

For additional tumor types, based on a retrospective series of Schrader et al (2016)²², which utilized a separate but similarly ascertained patient population at the same institution and identical methods of genotyping and variant interpretation, V_A was measured directly and is taken from Figure 3 of Schrader et al., while I_A was approximated as the number of discordant P/LPGV.

2. Comparison of Germline Data to Public Databases.

To assess association of specific variants and tumor phenotypes, population allele frequencies were extracted from the exome aggregation consortium (ExAC) data²³ accessible both as a download and through a browser²⁴ excluding cases derived from cancer patients as part of The Cancer Genome Atlas (TCGA). Further details of ExAC are available at <http://exac.broadinstitute.org/faq>. Data were stratified by European and Ashkenazi subsets^{23,25-27}. Comparisons of allele frequencies in cases of Ashkenazi ancestry were restricted to a control population of Ashkenazi ancestry²⁸. Allele frequencies were compared by Fisher Exact test in R version 3.3 using RStudio Version 0.99.903. Where proportions are presented, 95% confidence intervals were derived. Comparison of clinical variables in genetically define subsets (e.g. age, interval from diagnosis to genetic analysis) were compared by two sample t-test for independent or correlated samples, as appropriate. Comparisons of rates of incremental findings between subsets was by Fisher's Exact test.

3. Correlation of Germline Mutation Status and Mutational Load and Time to Metastases

In order to determine if there was an association between presence of germline P/LPGV and tumor burden of somatic mutations, a comparison was made between germline P/LPGV and total burden of somatic mutations. Burden of somatic mutations was defined as the number of single nucleotide variants and indels, and did not include copy number variants or silent mutations. In this global analysis, cases of germline mismatch repair gene mutations were included in the P/LPGV positive group, as well as somatic MLH1 promotor hypermethylation included in the P/LPGV negative group. Each case with a P/LPGV was given a numerical score representing the sum of the tumor P/LPGV's and compared to the scores of cases without P/LPGV by two sample t-test for independent samples, with two tailed p value. For the analysis of time to diagnosis we determined the average time between initial diagnosis of malignancy as recorded in the medical record and time to presentation with metastatic disease for tumor normal sequencing at our institution. Comparisons were by two sample t-test for independent samples, with two tailed p values

eBox. Ashkenazi Jewish and European founder mutations.

| Founder Mutations |
|---|
| <i>BRCA1</i> c.68_69delAG (p.Glu23Valfs*17) |
| <i>BRCA1</i> c.5266dupC (p.Gln1756Profs*74) |
| <i>BRCA2</i> c.5946delT (p.Ser1982Argfs*22), |
| <i>MSH2</i> c.1906G>C (p.Ala636Pro) |
| <i>MSH6</i> c.3959_3962delCAAG (p. Ala1320Glu fs*6) |
| <i>CHEK2</i> c.1100delC (p.Thr367Metfs*15) |
| <i>CHEK2</i> c.1283C>T (p.Ser428Phe) |
| <i>APC</i> c.3920T>A (p.Ile1307Lys) |
| <i>MUTYH</i> c.1187G>A (p.Gly396Asp) |
| <i>MUTYH</i> c. 536A>G (p.Tyr179Cys) |

eTable 1. Clinically actionable pathogenic or likely pathogenic variants in 182 patients.

| Gene | Transcript | Variant cDNA Change (c.) | Variant Amino Acid Change (p.) | Number of Cases With Variant |
|--------------|------------|--------------------------|--------------------------------|------------------------------|
| <i>APC</i> | NM_000038 | c.3920T>A | p.Ile1307Lys | 24 |
| <i>ATM</i> | NM_000051 | c.742C>T | p.Arg248* | 1 |
| <i>ATM</i> | NM_000051 | c.1027_1030delGAAA | p.Glu343Ilefs*2 | 1 |
| <i>ATM</i> | NM_000051 | c.1057_1058delTG | p.Cys353Serfs*5 | 1 |
| <i>ATM</i> | NM_000051 | c.1065+1G>T | | 1 |
| <i>ATM</i> | NM_000051 | c.2554C>T | p.Gln852* | 1 |
| <i>ATM</i> | NM_000051 | c.2849T>G | p.Leu950Arg | 1 |
| <i>ATM</i> | NM_000051 | c.3799G>T | p.Glu1267* | 1 |
| <i>ATM</i> | NM_000051 | c.5908 C>T | p.Gln1970* | 1 |
| <i>ATM</i> | NM_000051 | c.7775C>G | p.Ser2592Cys | 1 |
| <i>ATM</i> | NM_000051 | c.8786+1G>C | | 1 |
| <i>ATM</i> | NM_000051 | c.8879G>A | p.Trp2960* | 1 |
| <i>ATM</i> | NM_000051 | deletion exons 62-63 | | 4 |
| <i>BAP1</i> | NM_004656 | c.437+1G>T | | 1 |
| <i>BAP1</i> | NM_004656 | c.1983+1_1983+3delinsAT | | 1 |
| <i>BAP1</i> | NM_004656 | deletion exons 15-17 | | 1 |
| <i>BRCA1</i> | NM_007294 | c.68_69delAG | p.Glu23Valfs*17 | 3 |
| <i>BRCA1</i> | NM_007294 | c.181T>G | p.Cys61Gly | 1 |
| <i>BRCA1</i> | NM_007294 | c.427G>T | p.Glu143* | 1 |
| <i>BRCA1</i> | NM_007294 | c.4936delG | p.V1646Sfs*12 | 1 |
| <i>BRCA1</i> | NM_007294 | c.4986+5G>A | | 1 |
| <i>BRCA1</i> | NM_007294 | c.5266dupC | p.Gln1756Profs*74 | 6 |
| <i>BRCA1</i> | NM_007294 | del exon8 | | 1 |
| <i>BRCA2</i> | NM_000059 | c.25delC | p.Pro9Glnfs*16 | 1 |
| <i>BRCA2</i> | NM_000059 | c.793+1G>A | | 1 |
| <i>BRCA2</i> | NM_000059 | c.1189_1190insTTAG | p.Gln397Leufs*25 | 1 |
| <i>BRCA2</i> | NM_000059 | c.1754delA | p.Lys585Argfs*29 | 1 |
| <i>BRCA2</i> | NM_000059 | c.1813dupA | p.Ile605Asnfs*11 | 1 |
| <i>BRCA2</i> | NM_000059 | c.2047_2050delTCTC | p.Ser683Argfs*46 | 1 |
| <i>BRCA2</i> | NM_000059 | c.2094delA | p.Gln699Serfs*31 | 1 |
| <i>BRCA2</i> | NM_000059 | c.3215T>G | p.Leu1072* | 1 |
| <i>BRCA2</i> | NM_000059 | c.3922G>T | p.Glu1308* | 1 |
| <i>BRCA2</i> | NM_000059 | c.4131_4132insTGAGGA | p.Thr1378* | 1 |
| <i>BRCA2</i> | NM_000059 | c.4449delA | p.Asp1484Thrfs*2 | 1 |
| <i>BRCA2</i> | NM_000059 | c.4638delT | p.Phe1546Leufs*22 | 1 |
| <i>BRCA2</i> | NM_000059 | c.4827_4828delTG | p.Val1610Glyfs*4 | 1 |
| <i>BRCA2</i> | NM_000059 | c.5159C>A | p.Ser1720* | 1 |
| <i>BRCA2</i> | NM_000059 | c.5364dupC | p.Lys1789Glnfs*18 | 1 |
| <i>BRCA2</i> | NM_000059 | c.5614A>T | p.Lys1872* | 1 |
| <i>BRCA2</i> | NM_000059 | c.5946delT | p.Ser1982Argfs*22 | 18 |

| Gene | Transcript | Variant cDNA Change (c.) | Variant Amino Acid Change (p.) | Number of Cases With Variant |
|---------------|------------|--------------------------|--------------------------------|------------------------------|
| <i>BRCA2</i> | NM_000059 | c.6259delA | p.Arg2087Glufs*32 | 1 |
| <i>BRCA2</i> | NM_000059 | c.6282_6289delTTCACCTA | p.Ser2095Valfs*2 | 1 |
| <i>BRCA2</i> | NM_000059 | c.6591_6592delTG | p.Glu2198Asnfs*4 | 1 |
| <i>BRCA2</i> | NM_000059 | c.7879A>T | p.Ile2627Phe | 1 |
| <i>BRCA2</i> | NM_000059 | c.7978T>G | p.Tyr2660Asp | 1 |
| <i>BRCA2</i> | NM_000059 | c.8754+4A>G | | 1 |
| <i>BRCA2</i> | NM_000059 | c.9148C>T | p.Gln3050* | 1 |
| <i>BRCA2</i> | NM_000059 | c.9117G>A | p.Pro3039Pro | 1 |
| <i>BRCA2</i> | NM_000059 | c.9371A>T | p.Asn3124Ile | 1 |
| <i>BRCA2</i> | NM_000059 | c.9382C>T | p.Arg3128* | 1 |
| <i>BRCA2</i> | NM_000059 | c.9466C>T | p.Gln3156* | 1 |
| <i>BRIP1</i> | NM_032043 | c.2392C>T | p.Arg798* | 3 |
| <i>BRIP1</i> | NM_032043 | c.2400C>A | p.Tyr800* | 1 |
| <i>CDH1</i> | NM_004360 | c.715G>A | p.Gly239Arg | 1 |
| <i>CDH1</i> | NM_004360 | deletion exons 3-16 | | 1 |
| <i>CDKN2A</i> | NM_000077 | c.143C>G | p.Pro48Arg | 1 |
| <i>CDKN2A</i> | NM_000077 | c.176T>G | p.Val59Gly | 1 |
| <i>CDKN2A</i> | NM_000077 | c.457G>C | p.Asp153His | 1 |
| <i>CHEK2</i> | NM_007194 | c.16delG | p.Asp6Metfs*55 | 1 |
| <i>CHEK2</i> | NM_007194 | c.85C>T | p.Gln29* | 1 |
| <i>CHEK2</i> | NM_007194 | c.190G>A | p.Glu64Lys | 1 |
| <i>CHEK2</i> | NM_007194 | c.216T>G | p.Tyr72* | 1 |
| <i>CHEK2</i> | NM_007194 | c.283C>T | p.Arg95* | 1 |
| <i>CHEK2</i> | NM_007194 | c.444+1G>A | | 1 |
| <i>CHEK2</i> | NM_007194 | c.1100delC | p.Thr367Metfs*15 | 11 |
| <i>CHEK2</i> | NM_007194 | c.1111C>T | p.His371Tyr | 1 |
| <i>CHEK2</i> | NM_007194 | c.1283C>T | p.Ser428Phe | 11 |
| <i>CHEK2</i> | NM_007194 | deletion exons 9-10 | | 2 |
| <i>CHEK2</i> | NM_007194 | deletion exons 10-14 | | 1 |
| <i>FH</i> | NM_000143 | c.584T>C | p.Met195Thr | 1 |
| <i>FH</i> | NM_000143 | c.1394A>G | p.Tyr465Cys | 1 |
| <i>FH</i> | NM_000143 | deletion exon1 | | 1 |
| <i>FLCN</i> | NM_144997 | c.1285delC | p.His429Thrfs*39 | 1 |
| <i>MEN1</i> | NM_000244 | c.1322G>A | p.Trp441* | 1 |
| <i>MITF</i> | NM_000248 | c.952G>A | p.Glu318Lys | 2 |
| <i>MLH1</i> | NM_000249 | c.790+2T>C | | 1 |
| <i>MSH2</i> | NM_000251 | c.929T>G | p.Leu310Arg | 1 |
| <i>MSH2</i> | NM_000251 | c.942+3A>T | | 2 |
| <i>MSH2</i> | NM_000251 | c.1046C>G | p.Pro349Arg | 1 |
| <i>MSH2</i> | NM_000251 | c.1784T>G | p.Leu595Arg | 1 |
| <i>MSH2</i> | NM_000251 | c.1906G>C | p.Ala636Pro | 1 |
| <i>MSH2</i> | NM_000251 | deletion exons 1-8 | | 1 |

| Gene | Transcript | Variant cDNA Change (c.) | Variant Amino Acid Change (p.) | Number of Cases With Variant |
|---------------|--------------|--------------------------|--------------------------------|------------------------------|
| <i>MSH6</i> | NM_000179 | c.2731C>T | p.Arg911* | 1 |
| <i>MSH6</i> | NM_000179 | c.3261dupC | p.Phe1088Leufs*5 | 1 |
| <i>MSH6</i> | NM_000179 | c.3463C>T | p.Gln1155* | 1 |
| <i>MSH6</i> | NM_000179 | c.3959_3962delCAAG | p.Ala1320Glufs*6 | 1 |
| <i>MUTYH</i> | NM_001128425 | c.207delC | p.Arg70Glyfs*21 | 1 |
| <i>MUTYH</i> | NM_001128425 | c.536A>G | p.Tyr179Cys | 3 |
| <i>MUTYH</i> | NM_001128425 | c.821G>A | p.Arg274Gln | 2 |
| <i>MUTYH</i> | NM_001128425 | c.1156delC | p.Gln386Lysfs*22 | 1 |
| <i>MUTYH</i> | NM_001128425 | c.1187G>A | p.Gly396Asp | 5 |
| <i>MUTYH</i> | NM_001128425 | c.1214C>T | p.Pro405Leu | 1 |
| <i>MUTYH</i> | NM_001128425 | c.1240C>T | p.Gln414* | 1 |
| <i>MUTYH</i> | NM_001128425 | c.1356delA | p.Tyr453Ilefs*14 | 1 |
| <i>MUTYH</i> | NM_001128425 | c.1437_1439delGGA | p.Glu480del | 1 |
| <i>NBN</i> | NM_002485 | c.56delT | p.Leu19* | 1 |
| <i>NBN</i> | NM_002485 | c.657_661delACAAA | p.Lys219Asnfs*16 | 1 |
| <i>PALB2</i> | NM_024675 | c.3234T>A | p.Cys1078* | 1 |
| <i>PALB2</i> | NM_024675 | c.3323delA | p.Tyr1108Serfs*16 | 1 |
| <i>PALB2</i> | NM_024675 | c.3549C>G | p.Tyr1183* | 1 |
| <i>PALB2</i> | NM_024675 | deletion exon 11 | | 1 |
| <i>PALB2</i> | NM_024675 | duplication exon 13 | | 1 |
| <i>PMS2</i> | NM_000535 | c.137G>T | p.Ser46Ile | 1 |
| <i>PMS2</i> | NM_000535 | c.1605_1606del | p.Gln536Glyfs*5 | 1 |
| <i>RAD51D</i> | NM_001142571 | del exons 1-3 | | 1 |
| <i>SDHA</i> | NM_004168 | c.91C>T | p.Arg31* | 2 |
| <i>SDHB</i> | NM_003000 | c.587G>A | p.Cys196Tyr | 1 |
| <i>SDHB</i> | NM_003000 | c.640C>T | p.Gln214* | 1 |
| <i>VHL</i> | NM_000551 | c.154G>T | p.Glu52* | 1 |
| <i>VHL</i> | NM_000551 | c.499C>T | p.Arg167Trp | 1 |

eTable 2. Content of tumor type or syndrome-specific germline testing panels applied to cases according to family history and phenotype.

| | Breast-Ovarian | Breast | Colorectal | Ovarian | Pancreas | Polyposis | Renal | Paranganglioma |
|---------------|-----------------------|---------------|-------------------|----------------|-----------------|------------------|--------------|-----------------------|
| <i>APC</i> | | | X | | | X | | |
| <i>ATM</i> | X | X | | | X | | | |
| <i>BAP1</i> | | | | | | | X | X |
| <i>BRCA1</i> | X | X | | X | X | | | |
| <i>BRCA2</i> | X | X | | X | X | | | |
| <i>BMRP1A</i> | | | | | | | | |
| <i>BRIP1</i> | X | | | X | | | | |
| <i>CDH1</i> | | X | | | | | | |
| <i>CDK4</i> | | | | | X | | | |
| <i>CDKN2A</i> | | | | | X | | | |
| <i>CHEK2</i> | X | X | | | | | | |
| <i>EPCAM</i> | X | | X | X | X | | X | |
| <i>FH</i> | | | | | | | X | X |
| <i>FLCN</i> | | | | | | | X | X |
| <i>GREM1</i> | | | | | | | | |
| <i>MET</i> | | | | | | | X | X |
| <i>MITF</i> | | | | | | | X | X |
| <i>MLH1</i> | X | | X | X | X | | X | X |
| <i>MSH2</i> | X | | X | X | X | | X | X |
| <i>MSH6</i> | X | | X | X | X | | X | X |
| <i>MUTYH</i> | | | X | | | X | | |
| <i>PALB2</i> | X | X | | X | X | | | |

| | Breast-Ovarian | Breast | Colorectal | Ovarian | Pancreas | Polyposis | Renal | Paraganglioma |
|---------------|----------------|--------|------------|---------|----------|-----------|-------|---------------|
| <i>PMS2</i> | x | | x | x | x | | x | x |
| <i>POLD1</i> | | | | | | | | |
| <i>POLE</i> | | | | | | | | |
| <i>PTEN</i> | x | x | | | | | x | x |
| <i>RAD51C</i> | x | | | x | | | | |
| <i>RAD51D</i> | x | | | x | | | | |
| <i>SDHA</i> | | | | | | | x | x |
| <i>SDHB</i> | | | | | | | x | x |
| <i>SDHC</i> | | | | | | | x | x |
| <i>SDHD</i> | | | | | | | x | x |
| <i>SMAD4</i> | | | | | | | | |
| <i>STK11</i> | | | | | | | | |
| <i>TP53</i> | x | x | | | | | x | x |
| <i>TSC1</i> | | | | | | | x | x |
| <i>TSC2</i> | | | | | | | x | x |
| <i>VHL</i> | | | | | | | x | x |

Tumor syndrome listed in the headers for each column, and gene listed as the header for each row with “x” denoting inclusion in the syndrome-specific panel.

eTable 3. Results by tumor type, penetrance, and founder mutations in 205 cases with germline variants detected following tumor-normal analysis of 1040 cases.

| | No. of Cases | Tumor Type | | | | | | | | | | Total | Proportion of Total 1040 Cases | Proportion of Total Pathogenic Mutations |
|----------------------------------|--------------|------------|---------|--------|------------|-----------------|---------|-----------------|-----------------|-------|-------|-------|--------------------------------|--|
| | | Biliary | Bladder | Breast | Colorectal | Esophagogastric | Ovarian | Pancreatic | Prostate | Renal | Other | | | |
| 1040 total cases | 1040 | 27 | 16 | 101 | 65 | 34 | 19 | 176 | 362 | 140 | 100 | 1040 | 1.0 | |
| All Pathogenic Variants | 205 | 6 | 9 | 17 | 8 | 6 | 6 | 44 | 71 | 23 | 15 | 205 | 0.20 | 1.0 |
| Cases with Founder Mutations | 79 | 3 | 2 | 9 | 5 | 2 | 4 | 19 ^a | 26 ^a | 6 | 3 | 79 | 0.08 | 0.04 |
| High Penetrance | 97 | 4 | 8 | 4 | 2 | 4 | 6 | 21 | 31 | 11 | 6 | 97 | 0.09 | 0.47 |
| Cases with Founder Mutations | 33 | 1 | 2 | 2 | 1 | 2 | 4 | 8 ^a | 13 ^a | 0 | 0 | 33 | 0.03 | 0.16 |
| Moderate Penetrance | 52 | 0 | 0 | 11 | 3 | 1 | 0 | 10 | 19 | 5 | 3 | 52 | 0.05 | 0.25 |
| Cases with Founder Mutations | 21 | 0 | 0 | 7 | 2 | 0 | 0 | 4 | 4 | 3 | 1 | 21 | 0.02 | 0.10 |
| Low Penetrance | 33 | 2 | 0 | 0 | 3 | 0 | 0 | 8 | 12 | 4 | 4 | 33 | 0.03 | 0.16 |
| Cases with Founder Mutations | 25 | 2 | 0 | 0 | 2 | 0 | 0 | 7 ^a | 9 | 3 | 2 | 25 | 0.02 | 0.12 |
| Recessive Alleles | 8 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 3 | 1 | 1 | 8 | 0.01 | 0.04 |
| Cases with Founder Mutations | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Uncertain Clinical Actionability | 15 | 0 | 1 | 1 | 0 | 0 | 0 | 4 | 6 | 2 | 1 | 15 | 0.01 | 0.07 |
| Cases with Founder Mutations | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

^a Includes cases with 2 founder mutations

eTable 4. Incidence of findings not predicted by clinical guidelines (incremental findings) reflecting proportion attributed to founder mutations, and the proportion attributable to DNA repair mutations in the subset of advanced prostate cancer cases.

| | Tumor Type | | | | | | | | | | | Proportion (95% CI) |
|--|------------|------------|-------------|------------|-----------------|------------|-------------|--------------|-------------|------------|--------------|---------------------|
| | Biliary | Bladder | Breast | Colorectal | Esophagogastric | Ovarian | Pancreatic | Prostate | Renal | Other | Total | |
| Incremental clinically actionable | 4/6 | 3/8 | 3/15 | 5/8 | 1/5 | 1/6 | 20/39 | 44/62 | 12/20 | 8/13 | 101/182 | 0.56 (0.48-0.63) |
| Proportion of incremental clinically actionable cases | 0.67 | 0.38 | 0.20 | 0.63 | 0.20 | 0.17 | 0.51 | 0.71 | 0.60 | 0.62 | | |
| Confidence Interval (95%) | 0.30-0.90 | 0.14-0.69 | 0.07-0.45 | 0.31-0.86 | 0.04-0.63 | 0.03-0.56 | 0.36-0.66 | 0.59-0.80 | 0.39-0.78 | 0.36-0.82 | | |
| Incremental actionable cases with founder mutations subtracted | 2/6 | 3/8 | 1/15 | 3/8 | 1/5 | 0/6 | 8/39 | 27/62 | 6/20 | 6/13 | 57/182 | 0.31 (0.25-0.38) |
| Incremental clinically actionable cases without founder mutations and with DNA repair gene mutations subtracted from prostate cancer cases | 2/6 | 3/8 | 1/15 | 3/8 | 1/5 | 0/6 | 8/39 | 5/62 | 6/20 | 6/13 | 35/182 | 0.19 (0.14-0.26) |
| High Penetrance Cases (Incremental) | 2/4 | 3/8 | 1/3 | 0/2 | 0/4 | 0/5 | 4/19 | 12/29 | 3/11 | 2/6 | 27/91 | |
| Founder mutations subtracted | 2/4 | 3/8 | 1/3 | 0/2 | 0/4 | 0/5 | 4/19 | 10/29 | 3/11 | 2/6 | 25/91 | |
| DNA repair gene mutations subtracted from prostate cancer cases | 2/4 | 3/8 | 1/3 | 0/2 | 0/4 | 0/5 | 4/19 | 0/29 | 3/11 | 2/6 | 15/91 | |
| Moderate Penetrance Cases (Incremental) | 0 | 0 | 0/10 | 3/3 | 1/1 | 0 | 7/11 | 20/21 | 5/5 | 2/3 | 38/54 | |
| Founder mutations subtracted | 0 | 0 | 0/10 | 2/3 | 1/1 | 0 | 3/11 | 14/21 | 2/5 | 2/3 | 24/54 | |
| DNA repair gene mutations subtracted from prostate cancer cases | 0 | 0 | 0/10 | 2/3 | 1/1 | 0 | 3/11 | 2/21 | 2/5 | 2/3 | 12/54 | |
| Low Penetrance Cases (Incremental) | 2/2 | 0 | 2/2 | 2/3 | 0 | 1/1 | 9/9 | 12/12 | 4/4 | 4/4 | 36/37 | |
| Founder mutations subtracted | 0/2 | 0 | 0/2 | 1/3 | 0 | 0/1 | 1/9 | 3/12 | 1/4 | 2/4 | 8/37 | |
| DNA repair gene mutations subtracted from prostate cancer cases | 0/2 | 0 | 0/2 | 1/3 | 0 | 0/1 | 1/9 | 3/12 | 1/4 | 2/4 | 8/37 | |

This table reflects the total number of incremental clinically actionable mutations, and the number of those mutations minus founder mutations in each tumor category and categorized by penetrance. For the subset of prostate cancer cases only, a further subtraction is made for cases with variants in DNA repair genes associated with advanced prostate cancer. In the analysis of incremental findings not predicted by clinical guidelines, when accounting for cases with both an actionable incremental variant and one that was not actionable or incremental, then those cases were counted as having an actionable incremental finding. Decision rules for determination of penetrance levels, clinically actionable status and incremental status for cases with more than one variant are specified in eAppendix 5.

eTable 5. Therapeutic implications of secondary germline findings.

| Case No. | Tumor Type | Gene | Variants | Incremental Status | Targeted Treatment Based on Germline Analysis | Discussion Content of Targeted Therapeutic Options |
|----------|----------------------------------|--------------|---------------------------|--------------------|---|--|
| R164 | Ampullary Carcinoma ^a | <i>BRCA2</i> | c.25delC (p.Pro9Glnfs*16) | Y | N | In a patient with ampullary carcinoma, after documentation of a <i>BRCA2</i> mutation not suspected by family history, there was a discussion about potential benefit of chemotherapy in the context of recurrent disease, especially with a platinum-based therapy, as well as a new discussion of off label use of PARP inhibitors. |
| R155 | Biliary Cancer ^a | <i>BRCA2</i> | c.3215T>G (p.Leu1072*) | Y | Y (Olaparib) | In a patient with biliary cancer, after documentation of a <i>BRCA2</i> mutation not suspected by family history, there was a discussion of use of DNA damagng agents and off label PARP inhibitors. There was a discussion of response to irinotecan in <i>BRCA2</i> -deficient hepatobiliary cancers. Subsequently, patient received Olaparib in the context of clinical trial at NIH. |
| R201 | Esophagogastric Carcinoma | <i>ATM</i> | c.3799G>T (p.Glu1267*) | Y | N | In a patient with esophagogastric carcinoma, after documentation of an <i>ATM</i> mutation not suspected by family history, there was new discussion of patient's eligibility for an open clinical trial of ATR kinase inhibitor; patient declined participation in the trial. |
| R173 | Pancreatic Cancer | <i>CHEK2</i> | c. 1111C>T (p.His371Tyr) | Y | N | In a patient with pancreatic cancer, after documentation of a <i>CHEK2</i> mutation not suggested by family history, there was new discussion of off label or protocol use of PARP inhibitors. |
| R188 | Pancreatic Cancer | <i>ATM</i> | deletion exons 62-63 | Y | N | In a patient with pancreatic cancer, after documentation of an <i>ATM</i> mutation not suspected by family history, there was new discussion of PARP inhibitors use in the context of clinical trials, as well as endorsement of the platinum-based therapy in progress at the time. |
| R190 | Pancreatic Cancer | <i>BRCA2</i> | c.793+1G>A | Y | Y (Olaparib) | In a patient with pancreatic cancer, after documentation of a <i>BRCA2</i> mutation not suspected by family history, there was new discussion of off label or protocol use of PARP inhibitors, as well as likely impact of the germline mutational status on observed favorable response to prior platinum-based chemotherapy. Olaparib was added to the treatment later. |

| Case No. | Tumor Type | Gene | Variants | Incremental Status | Targeted Treatment Based on Germline Analysis | Discussion Content of Targeted Therapeutic Options |
|----------|--------------------------------|--------------|--------------------------------|--------------------|---|--|
| R26 | Pancreatic Cancer ^a | <i>BRCA2</i> | c. 5614A>T (p. Lys1872*) | Y | N | In a patient with pancreatic cancer, after documentation of a <i>BRCA2</i> mutation not suspected by family history, there was new discussion of off label or protocol use of PARPi as well as discussion of the continuation of platinum-based therapies and continuation of FOLFIRINOX (containing a platinum compound) in the setting of <i>BRCA2</i> mutation. |
| R51 | Prostate Cancer | <i>BRCA2</i> | c.5946delT (p.Ser1982Argfs*22) | Y | N | In a patient with prostate cancer, after documentation of a <i>BRCA2</i> mutation not suspected by family history, there was discussion of starting off label or protocol treatment with PARP inhibitors. |
| R131 | Pancreatic Cancer | <i>BRCA2</i> | c.5946delT (p.Ser1982Argfs*22) | N | Y (Olaparib) | In a patient with pancreatic cancer, after documentation of a <i>BRCA2</i> mutation suggested by family history, there was discussion of platinum-based therapy and off label or protocol use of PARP inhibitors. Olaparib therapy was initiated. |
| R144 | Pancreatic Cancer | <i>BRCA1</i> | c.181T>G (p.Cys61Gly) | N | Y (Platinum re-sensitization, Olaparib) | In a patient with pancreatic cancer, after documentation of a <i>BRCA1</i> mutation suggested by family history, there was discussion of potential use of off label or protocol use of PARP inhibitors, as well as re-sensitization to platinum-based chemotherapy. Cisplatin chemotherapy was initiated, with later use of off label Olaparib. |
| R95 | Prostate Cancer | <i>CHEK2</i> | c.1100delC (p.Thr367Metfs*15) | Y | N | In a patient with prostate cancer, after documentation of a <i>CHEK2</i> mutation not suspected by family history (and somatic <i>BRCA2</i> mutation), there was discussion of potential use of alkylating agents and off label use of PARP inhibitors. |
| R120 | Prostate Cancer | <i>ATM</i> | c.7775C>G (p.Ser2592Cys) | Y | Y (Carboplatin) | In a patient with prostate cancer, after documentation of an <i>ATM</i> mutation not suspected by family history, there was new discussion of potential use of alkylating agents and off label use of PARP inhibitors. Platinum-based chemotherapy was subsequently added to androgen deprivation therapy. |

| Case No. | Tumor Type | Gene | Variants | Incremental Status | Targeted Treatment Based on Germline Analysis | Discussion Content of Targeted Therapeutic Options |
|----------|------------------------------|------------------------------|---|--------------------|---|---|
| R132 | Prostate Cancer | <i>PALB2</i> | deletion exon 11 | Y | N | In a patient with prostate cancer, after documentation of a <i>PALB2</i> mutation not suspected by family history, there was new discussion of potential use of alkylating agents and PARP inhibitors, including Olaparib, for DNA repair-deficient prostate cancer resistant to standard therapies. |
| R161 | Prostate Cancer | <i>CHEK2</i> <i>FLCN</i> | c.1100delC (p.Thr367Metfs*15), c.1285delC (p.His429Thrfs*39) | Y N | N | In a patient with prostate cancer, after documentation of a <i>CHEK2</i> mutation not suspected by family history, there was discussion of off label or protocol treatment with PARP inhibitors, as well as use of alkylating agents. |
| R205 | Prostate Cancer | <i>BRCA2</i> | c.2094delA (p.Gln699Serfs*31) | Y | Y (Carboplatin) | In a patient with prostate cancer, after documentation of a <i>BRCA2</i> mutation not suggested by family history, there was discussion of alkylating agents and off label or protocol use of a PARP inhibitor at the time of progression of disease. Platinum-based chemotherapy was initiated shortly thereafter. |
| R79 | Prostate Cancer ^a | <i>BRCA2</i> | c.7879A>T (p.Ile2627Phe) | Y | N | In a patient with prostate cancer, after documentation of a <i>BRCA2</i> mutation not suggested by family history, there was new discussion of off label or protocol use of a PARP inhibitor, including Olaparib. |
| R166 | Prostate Cancer ^a | <i>PMS2</i> | c.137G>T (p.Ser46Ile) | Y | N | In a patient with prostate cancer, after documentation of a <i>PMS2</i> mutation not suspected by family history and investigation of the associated mismatch repair deficiency, there was new discussion of potential use of immune checkpoint therapy, off label or in the context of clinical trial at the time of the discussion. |
| R179 | Prostate Cancer | <i>BRCA2</i> <i>CHEK2</i> | c.5946delT (p.Ser1982Argfs*22) c.1283C>T (p.Ser428Phe) | N Y | N | In a patient with prostate cancer, after identification of both a <i>BRCA2</i> mutation suggested by family history, and a <i>CHEK2</i> mutation not suspected by family history, there was discussion of potential use of off label PARP inhibitors in case of resistance to hormone-based therapy. |
| R14 | Ovarian Cancer | <i>PALB2</i> | duplication exon 13 | N | N ^b | In a patient with ovarian cancer, after identification of a <i>PALB2</i> mutation suggested by family history, there was new discussion of eligibility for a Phase I clinical trial involving PARPi in combination with an <i>ATM</i> inhibitor. |

| Case No. | Tumor Type | Gene | Variants | Incremental Status | Targeted Treatment Based on Germline Analysis | Discussion Content of Targeted Therapeutic Options |
|----------|------------------------------|--------------|--|--------------------|---|--|
| R48 | Pancreatic Cancer | <i>BRCA1</i> | c.68_69delAG (p.Glu23Valfs*17) | N | N | In a patient with pancreatic cancer, after documentation of a <i>BRCA1</i> mutation suggested by family history, there was discussion of potential use of off label or protocol use of PARP inhibitors, as well as likely association of patient's favorable response to FOLFIRINOX (containing a platinum compound) with the <i>BRCA1</i> mutation. |
| R57 | Prostate Cancer | <i>BRCA2</i> | c.1189_1190insTTAG (p.Gln397Leufs*25) | N | N | In a patient with prostate cancer, after documentation of a <i>BRCA2</i> mutation suggested by family history, there was new discussion of off label or protocol use of a PARP inhibitor at the time of progression of disease. |
| R60 | Prostate Cancer ^a | <i>BRCA2</i> | c.5946delT (p.Ser1982Argfs*22) | N | N | In a patient with prostate cancer, after documentation of a <i>BRCA2</i> mutation suggested by family history, there was new discussion of off label or protocol use of a PARP inhibitor (Olaparib). |
| R61 | Breast Carcinoma | <i>BRCA2</i> | c.6282_6289delTTCACCT A (p.Ser2095Valfs*2) | N | N | In a patient with breast cancer, after documentation of a <i>BRCA2</i> mutation suggested by family history, there was discussion of potential protocol use of PARPi. |
| R63 | Prostate Cancer ^a | <i>BRCA2</i> | c.5946delT (p.Ser1982Argfs*22) | N | N ^b | In a patient with prostate cancer, after documentation of a <i>BRCA2</i> mutation consistent with family history, there was discussion of off label or protocol treatment with PARPi, as well as use of platinum-based chemotherapy at the time of progression of disease. |
| R75 | Prostate Cancer | <i>BRCA2</i> | c.5946delT (p.Ser1982Argfs*22) | N | N | In a patient with prostate cancer, after documentation of a <i>BRCA2</i> mutation suggested by family history, there was new discussion of potential use of alkylating agents and off label or protocol use of a PARP inhibitor. |
| R76 | Prostate Cancer ^a | <i>BRCA2</i> | c.5364dupC (p.Lys1789Glnfs*18) | N | N | In a patient with prostate cancer, after documentation of a <i>BRCA2</i> mutation suggested by family history, there was new discussion of off label or protocol use of PARP inhibitors (including Olaparib and Rucaparib), as well as use of platinum-based chemotherapy in a setting of a <i>BRCA2</i> mutation in case of disease progression. |

| Case No. | Tumor Type | Gene | Variants | Incremental Status | Targeted Treatment Based on Germline Analysis | Discussion Content of Targeted Therapeutic Options |
|----------|-----------------------------|--------------|-----------------------------------|--------------------|---|---|
| R84 | Esophagogastric Carcinoma | <i>BRCA2</i> | c.5946delT (p.Ser1982Argfs*22) | N | N ^b | In a patient with esophagogastric carcinoma, after documentation of a <i>BRCA2</i> mutation suggested by family history, there was discussion of continuation of EOX (epirubicin, oxaliplatin, capecitabine) chemotherapy containing a platinum compound in the setting of <i>BRCA2</i> mutation, as well as off label or protocol use of PARP inhibitors (Olaparib, Neratinib, Rucaparib). |
| R62 | Prostate Cancer | <i>ATM</i> | c.742C>T (p.Arg248*) | Y | Y (Olaparib) | In a patient with prostate cancer, after documentation of an <i>ATM</i> mutation not suggested by family history, there was discussion of use of a PARP inhibitor (Olaparib) at the time of progression of disease. Olaparib was subsequently added to treatment shortly thereafter, following insurance approval. |
| R112 | Biliary Cancer ^a | <i>BRCA2</i> | c.4131_4132insTGAGGA (p.Thr1378*) | N | N ^b | In a patient with biliary cancer, after documentation of a <i>BRCA2</i> mutation suggested by family history, there was discussion of off label and protocol use of PARP inhibitors, re-exposure to a platinum-based chemotherapy used in the past, and other platinum-based chemotherapy options. |
| R71 | Prostate Cancer | <i>ATM</i> | c.8786+1G>C | Y | Y (Olaparib) | In a patient with prostate cancer, after documentation of an <i>ATM</i> mutation not suggested by family history, there was discussion of potential use of targeted treatments based on molecular profile, including PARP inhibitors (Olaparib) at the time of progression of disease. Olaparib was subsequently added to treatment. |
| R140 | Prostate Cancer | <i>BRCA2</i> | c.3922G>T (p.Glu1308*) | N | N | In a patient with prostate cancer, after identification of <i>BRCA2</i> mutation suggested by family history, there was discussion of off label PARP inhibitors use. |
| R94 | Prostate Cancer | <i>BRCA2</i> | c. 5159C>A (p.Ser1720*) | N | Y (Olaparib, ATR kinase inhibitor) | In a patient with prostate cancer, after documentation of a <i>BRCA2</i> mutation suggested by family history, there was new discussion of off label or protocol use of a PARP inhibitor. Patient subsequently received Olaparib and ATR kinase inhibitor in the context of a clinical trial. |

| Case No. | Tumor Type | Gene | Variants | Incremental Status | Targeted Treatment Based on Germline Analysis | Discussion Content of Targeted Therapeutic Options |
|----------|--------------------------------|--------------|---------------------------------|--------------------|---|---|
| R154 | Prostate Cancer | <i>BRCA2</i> | c.5946delT (p.Ser1982Argfs*22) | N | Y (Olaparib) | In a patient with prostate cancer, after documentation of a <i>BRCA2</i> mutation suggested by family history, there was new discussion of off label or protocol use of PARP inhibitors (including Olaparib). Olaparib was subsequently added to therapy. |
| R157 | Pancreatic Cancer | <i>BRCA1</i> | c.5266dupC (p.Gln1756Profs*74) | N | N | In a patient with pancreatic cancer, after documentation of a <i>BRCA1</i> mutation suggested by family history, there was discussion of use of platinum-based combination chemotherapies (including cisplatin/gemcitabine, FOLFIRINOX or other multidrug therapies including platinum compounds) as well as off label and protocol use of PARP inhibitors (including clinical trial with gemcitabine, cisplatin, with or without Veliparib). |
| R159 | Prostate Cancer | <i>ATM</i> | c.8879G>A (p.Trp2960*) | N | Y (Carboplatin, Rucaparib) | In a patient with prostate cancer, after documentation of an <i>ATM</i> mutation consistent with family history, there was discussion of use of platinum-based chemotherapy (carboplatin, docetaxel) or other topoisomerase active chemotherapy agents (mitoxantrone, etoposide, camptothecins) in the context of an <i>ATM</i> -deficient tumor, as well as off label and protocol use of PARP inhibitors. Platinum-based combination chemotherapy was subsequently initiated, followed later on by Rucaparib therapy. |
| R177 | Prostate Cancer | <i>BRCA1</i> | c.5266dupC (p.Gln1756Profs*74) | N | N ^b | In a patient with prostate cancer, after documentation of a <i>BRCA1</i> mutation suggested by family history, there was discussion of off label or protocol use of PARP inhibitors. |
| R183 | Pancreatic Cancer ^a | <i>BRCA2</i> | c.6259delA (p.Arg2087Glu fs*32) | N | N | In a patient with pancreatic cancer, after documentation of a <i>BRCA2</i> mutation suggested by family history, there was discussion of platinum-based therapy (e.g. cisplatin/gemcitabine, FOLFIRINOX), and off label or protocol use of PARP inhibitors, including a clinical trial of gemcitabine, cisplatin with or without Veliparib. |
| R200 | Pancreatic Cancer ^a | <i>BRCA1</i> | c.5266dupC (p.Gln1756Profs*74) | N | Unknown | In a patient with pancreatic cancer, after documentation of a <i>BRCA1</i> mutation suggested by family history, there was discussion of impact on future treatments, while endorsing continuation of a platinum-based treatment. |

^a Cases with second primary tumor

^b In these cases, targeted therapy was administered based on germline findings consistent with results, but performed separately from genomic testing in this study. These cases were not counted towards the total number of cases with treatment change due to secondary germline findings. Incremental status refers to germline test results which are not predicted (Y) or predicted (N) by clinical guidelines.

EOX refers to Eribulin, Oxaliplatin, and Capecitabine (Xeloda).

PARPi refers to PARP inhibitors.

FOLFRINOX refers to the Leucovorin, Fluorouracil, Irinotecan, and Oxaliplatin regimen.

eTable 6. Incidence of pathogenic or likely pathogenic genetic variants and incremental clinically actionable findings in Stage IV and Stage I-III cancer cases in the entire cohort and in the subsets of colon, pancreatic, prostate, renal, and all other cancer cases.

| | Number of Cases by Mutational Status | | | N (%) Pathogenic Variants | N (%) Clinically Actionable Mutations | N Incremental Clinically Actionable (% of Clinically Actionable) |
|----------------------|--------------------------------------|-----------------------------|-----------------------------|---------------------------|---------------------------------------|--|
| | N Total Cases | Cases with Negative Results | Cases with Positive Results | | | |
| All Cases | 1040 | 835 | 205 | 205/1040 (19.7) | 182/1040 (17.5%) | 101/182 (55.5%) |
| All Stage IV | 846 | 657 | 189 | 189/846 (22.3%) | 166/846 (19.6%) | 93/166 (56%) |
| All Stage 0-III | 194 | 178 | 16 | 16/194 (8.3%) | 16/194 (8.3%) | 8/16 (50%) |
| Colon | 65 | 57 | 8 | 8/65 (12.3%) | 8/65 (12.3%) | 5/8 (62.5%) |
| Colon Stage IV | 54 | 48 | 6 | 6/54 (11.1%) | 6/54 (11.1%) | 5/6 (83.3%) |
| Colon Stage I-III | 11 | 9 | 2 | 2/11 (18.2%) | 2/11 (18.2%) ^a | 0/2 (0%) |
| Pancreas | 176 | 132 | 44 | 44/176 (25%) | 39/176 (22.1%) | 20/39 (51.3%) |
| Pancreas Stage IV | 118 | 79 | 39 | 39/118 (33.1%) | 34/118 (28.8%) | 18/34 (52.9%) |
| Pancreas Stage I-III | 58 | 53 | 5 | 5/58 (8.6%) | 5/58 (8.6%) | 2/5 (40%) |
| Prostate | 362 | 291 | 71 | 71/362 (19.6%) | 62/362 (17.1%) | 44/62 (71%) |
| Prostate Stage IV | 308 | 240 | 68 | 68/308 (22.1%) | 59/308 (19.2%) | 41/59 (69.5%) |
| Prostate I-III | 54 | 51 | 3 | 3/54 (5.6%) | 3/54 (5.6%) | 3/3 (100%) |
| Renal | 140 | 117 | 23 | 23/140 (16.4%) | 20/140 (14.3%) | 12/20 (60%) |
| Renal Stage IV | 122 | 100 | 22 | 22/122 (18%) | 19/11 (15.6%) | 12/19 (63.2%) |
| Renal Stage I-III | 18 | 17 | 1 | 1/18 (5.6%) | 1/18 (5.6%) | 0/1 (0%) |

| | Number of Cases by Mutational Status | | | N (%) Pathogenic Variants | N (%) Clinically Actionable Mutations | N Incremental Clinically Actionable (% of Clinically Actionable) |
|--|--------------------------------------|-----------------------------|-----------------------------|---------------------------|---------------------------------------|--|
| | N Total Cases | Cases with Negative Results | Cases with Positive Results | | | |
| Other (All Except Colon, Pancreas, Prostate, Renal) | 297 | 238 | 59 | 59/297 (19.9%) | 53/297 (17.8%) | 20/53 (37.7%) |
| Other Stage IV | 244 | 190 | 54 | 54/244 (22.1%) | 48/244 (19.7%) | 17/48 (35.4%) |
| Other Stage 0-III | 53 | 48 | 5 | 5/53 (9.4%) | 5/53 (9.4%) | 3/5 (60%) |

^a Both colon cancer cases with Stage I-III disease had *MSH2* mutations, associated with Lynch Syndrome

Three bladder tumors were stage 0, hence their inclusion in the “Other” and “All Cases” categories

eTable 7. Findings incremental to phenotype based guidelines, demonstrating proportion of these attributable to founder mutations and DNA repair genes associated with prostate cancer, in subsets of the cohort stratified by self identified Ashkenazi (n=68) or non-Ashkenazi (n=124) ancestry.

| | Tumor Type | | | | | | | | | | Total |
|---|------------|---------|--------|------------|-----------------|---------|------------|----------|-------|-------|-------|
| | Biliary | Bladder | Breast | Colorectal | Esophagogastric | Ovarian | Pancreatic | Prostate | Renal | Other | |
| All Pathogenic Variants (n=205) | 6 | 9 | 17 | 8 | 6 | 6 | 44 | 71 | 23 | 15 | 205 |
| Cases with Pathogenic Variants with Known Ethnicity/Religion (n=192) | 5 | 9 | 16 | 6 | 6 | 6 | 43 | 65 | 22 | 14 | 192 |
| Ashkenazi Jewish, n=68 | 2 | 4 | 3 | 4 | 2 | 3 | 19 | 25 | 2 | 4 | 68 |
| Incremental findings (cases) | 1/2 | 2/4 | 1/3 | 3/4 | 0/2 | 1/3 | 12/19 | 16/25 | 2/2 | 2/4 | 40/68 |
| Incremental clinically actionable (minus Recessive and Uncertain) | 1/2 | 2/4 | 1/3 | 2/4 | 0/2 | 1/3 | 11/19 | 13/25 | 2/2 | 1/4 | 34/68 |
| Incremental clinically actionable minus <i>MUTYH</i> and <i>CHEK2</i> founder mutations | 1/2 | 2/4 | 1/3 | 2/4 | 0/2 | 1/3 | 9/19 | 12/25 | 2/2 | 1/4 | 31/68 |
| Incremental clinically actionable minus all (<i>MUTYH</i> , <i>CHEK2</i> and all Ashkenazi Jewish) founder mutations | 0/2 | 2/4 | 0/3 | 1/4 | 0/2 | 0/3 | 0/19 | 1/25 | 1/2 | 0/4 | 5/68 |

| | Tumor Type | | | | | | | | | | Total |
|--|------------|---------|--------|------------|-----------------|---------|------------|----------|-------|-------|--------|
| | Biliary | Bladder | Breast | Colorectal | Esophagogastric | Ovarian | Pancreatic | Prostate | Renal | Other | |
| Incremental clinically actionably minus all (<i>MUTYH</i> , <i>CHEK2</i> and all Ashkenazi Jewish) founder mutations and with DNA repair gene mutations subtracted from prostate cancer cases | 0/2 | 2/4 | 0/3 | 1/4 | 0/2 | 0/3 | 0/19 | 0/25 | 1/2 | 0/4 | 4/68 |
| Non-Ashkenazi Jewish, n=124 | 3 | 5 | 13 | 2 | 4 | 3 | 24 | 40 | 20 | 10 | 124 |
| Incremental findings (cases) | 2/3 | 2/5 | 3/13 | 1/2 | 2/4 | 0/3 | 12/24 | 33/40 | 12/20 | 7/10 | 74/124 |
| Incremental clinically actionable (minus Recessive and Uncertain) | 2/3 | 1/5 | 2/13 | 1/2 | 1/4 | 0/3 | 8/24 | 27/40 | 9/20 | 6/10 | 58/124 |
| Incremental clinically actionable minus <i>MUTYH</i> and <i>CHEK2</i> founder mutations | 1/3 | 1/5 | 1/13 | 1/2 | 1/4 | 0/3 | 8/24 | 22/40 | 5/20 | 6/10 | 46/124 |
| Incremental clinically actionably minus all (<i>MUTYH</i> , <i>CHEK2</i>) founder mutations and with DNA repair gene mutations subtracted from prostate cancer cases | 1/3 | 1/5 | 1/13 | 1/2 | 1/4 | 0/3 | 8/24 | 5/40 | 5/20 | 6/10 | 29/124 |

eTable 8. Allele frequency comparisons for variants detected in cases and public controls, matched by Ashkenazi or non-Ashkenazi ancestry.

| Tumor Type | Gene | Variant | Frequency in non-Ashkenazi | Ca-Non-Ashkenazi | ExAC Non-Finnish Europeans | P | Frequency in Ashkenazi | NonIBD-Ashkenazi | P | Frequency |
|------------|--------------|-------------------------------|----------------------------|------------------|----------------------------|-----------|------------------------|------------------|--------|-------------|
| Prostate | CHEK2 | c.1100delC (p.Thr367Metfs*15) | 3/191 | | | | 1/73 | 11/4352 | | 0/98 |
| | CHEK2 | c.190G>A (p.Glu64Lys) | 1/191 | | | | 0/73 | - | | 0/98 |
| | CHEK2 | c.470T>C (p.Ile157Thr) | 4/191 | | | | 0/73 | 3/4356 | | 1/98 |
| | CHEK2 | c.85C>T (p.Gln29*) | 1/191 | | | | 0/73 | - | | 0/98 |
| | CHEK2 | c.1283C>T (p.Ser428Phe) | 0/191 | | | | 1/73 | 45/4350 | | 0/98 |
| | TOTAL | TOTAL | 9/191 | 9/382 | 406/53707 | 0.00026 | 2/73 | 59/4354 | 1 | 1/98 |
| | MUTYH | c.536A>G (p.Tyr179Cys) | 2/191 | | | | 0/73 | - | | 0/98 |
| | MUTYH | c.1214C>T (p.Pro405Leu) | 1/191 | | | | 0/73 | - | | 0/98 |
| | MUTYH | c.821G>A (p.Arg274Gln) | 1/191 | | | | 0/73 | - | | 0/98 |
| | MUTYH | c.1156delC (p.Gln386Lysfs*22) | 1/191 | | | | 0/73 | - | | 0/98 |
| | TOTAL | TOTAL | 5/191 | 5/382 | 23/53470 | 0.0000016 | 0/73 | NA | NA | 0/98 |
| Pancreatic | CHEK2 | c.1100delC (p.Thr367Metfs*15) | 0/85 | | | | 2/60 | 11/4352 | | 0/31 |
| | CHEK2 | c.470T>C (p.Ile157Thr) | 2/85 | | | | 0/60 | 3/4356 | | 0/31 |
| | CHEK2 | c.1111C>T (p.His371Tyr) | 1/85 | | | | 0/60 | - | | 0/31 |
| | CHEK2 | c.283C>T (p.Arg95*) | 1/85 | | | | 0/60 | - | | 0/31 |
| | CHEK2 | c.1283C>T (p.Ser428Phe) | 0/85 | | | | 2/60 | - | | 0/31 |
| | TOTAL | TOTAL | 4/85 | 4/170 | 391/53334 | 0.039 | 4/60 | 14/4354 | 0.0013 | 0/31 |

| Tumor Type | Gene | Variant | Frequency in non-Ashkenazi | Ca-Non-Ashkenazi | ExAC Non-Finnish Europeans | P | Frequency in Ashkenazi | NonIBD-Ashkenazi | P | Frequency |
|------------|--------------|-------------------------------|----------------------------|------------------|----------------------------|-------|------------------------|------------------|---|-------------|
| Renal | <i>CHEK2</i> | c.470T>C (p.Ile157Thr) | 1/94 | | | | 0/16 | 3/4356 | | 0/30 |
| | <i>CHEK2</i> | c.1100delC (p.Thr367Metfs*15) | 2/94 | | | | 0/16 | 11/4352 | | 0/30 |
| | <i>CHEK2</i> | Deletion exons 9-10 | 1/94 | | | | 0/16 | - | | 0/30 |
| | <i>CHEK2</i> | c.1283C>T (p.Ser428Phe) | 0/94 | | | | 0/16 | 45/4350 | | 1/30 |
| | <i>CHEK2</i> | c.216T>G (p.Tyr72*) | 0/94 | | | | 1/16 | - | | 0/30 |
| | TOTAL | TOTAL | 4/94 | 4/188 | 388/53284 | 0.052 | 1/16 | 59/4354 | 1 | 1/30 |

The recurring *CHEK2*1100delC* (p.Thr367Metfs*15) mutation was included in this analysis because its population frequency of 0.3% in the source population of the cases²⁹ is closely comparable to the 0.3% population frequency in the control Exome Aggregation Consortium (ExAC) non-Finnish European population without cancer cases from The Cancer Genome Atlas (TCGA). For this association analysis, the *CHEK2* c.470T>C (p.Ile157Thr) was also included.

Frequency in non-Ashkenazi refers to allele frequency cases in the series who are not of self-reported Ashkenazi Jewish ancestry. Ca-Non-Ashkenazi refers to control frequency of the same mutation²³ minus cancer cases from TCGA^{23,25-27}. ExAC Non-Finnish European refers to the non-Finnish European subset of ExAC. Frequency in Ashkenazi refers to allele frequency of variants in patients in this series who self-reported Ashkenazi Jewish ancestry. NonIBD-Ashkenazi refers to allele frequency in a control population of Ashkenazi Jewish ancestry²⁸. P values refer to allele frequency comparisons by Fisher Exact 2-sided binomial test in R version 3.3 using RStudio Version 0.99.903. Where proportions are presented, 95% confidence intervals were derived. Comparison of clinical variables in genetically defined subsets (e.g. age, interval from diagnosis to genetic analysis) were compared by two sample t-test for independent or correlated samples, as appropriate. Comparisons of rates of incremental findings between subsets was by Fisher's Exact test.

NA indicates non-applicable.

eReferences. References supporting the eAppendices and eTables.

1. Cibulskis K, Lawrence MS, Carter SL, et al. Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. *Nature biotechnology*. 2013;31(3):213-219.
2. McKenna A, Hanna M, Banks E, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome research*. 2010;20(9):1297-1303.
3. Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res*. 2002;30(12):e57.
4. Cancer Genetics Program. 2015; <http://university.asco.org/cancer-genetics-program>. Accessed 10/19, 2016.
5. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Gastric Cancer. https://www.nccn.org/professionals/physician_gls/pdf/gastric.pdf.
6. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Pancreatic Adenocarcinoma. https://www.nccn.org/professionals/physician_gls/pdf/pancreatic.pdf. Accessed 12/15/2016, 2016.
7. Schmidt LS, Linehan WM. Genetic predisposition to kidney cancer. *Seminars in oncology*. 2016;43(5):566-574.
8. Lener MR, Scott RJ, Kluzniak W, et al. Do founder mutations characteristic of some cancer sites also predispose to pancreatic cancer? *International journal of cancer*. 2016;139(3):601-606.
9. Potrony M, Puig-Butille JA, Aguilera P, et al. Prevalence of MITF p.E318K in Patients With Melanoma Independent of the Presence of CDKN2A Causative Mutations. *JAMA dermatology*. 2016;152(4):405-412.
10. Ghiorzo P, Pastorino L, Queirolo P, et al. Prevalence of the E318K MITF germline mutation in Italian melanoma patients: associations with histological subtypes and family cancer history. *Pigment cell & melanoma research*. 2013;26(2):259-262.
11. Ramus SJ, Song H, Dicks E, et al. Germline Mutations in the BRIP1, BARD1, PALB2, and NBN Genes in Women With Ovarian Cancer. *J Natl Cancer Inst*. 2015;107(11).
12. Rafnar T, Gudbjartsson DF, Sulem P, et al. Mutations in BRIP1 confer high risk of ovarian cancer. *Nat Genet*. 2011;43(11):1104-1107.
13. Tung N, Domchek SM, Stadler Z, et al. Counselling framework for moderate-penetrance cancer-susceptibility mutations. *Nat Rev Clin Oncol*. 2016;13(9):581-588.
14. Easton DF, Pharoah PD, Antoniou AC, et al. Gene-panel sequencing and the prediction of breast-cancer risk. *The New England journal of medicine*. 2015;372(23):2243-2257.
15. NCCN. Genetic/Familial High-Risk Assessment: Breast and Ovarian (Version 1.2017-September 19, 2016). https://www.nccn.org/professionals/physician_gls/pdf/genetics_screening.pdf. Accessed September 23, 2016.

16. NCCN. Genetic/Familial High-Risk Assessment: Colorectal (Version 2.2016 - September 26, 2016).
https://www.nccn.org/professionals/physician_gls/pdf/genetics_colon.pdf. Accessed September 23, 2016.
17. Menko FH, van Steensel MAM, Giraud S, et al. Birt-Hogg-Dubé syndrome: diagnosis and management. *The Lancet Oncology*. 2009;10(12):1199-1206.
18. Pilarski R, Cebulla CM, Massengill JB, et al. Expanding the clinical phenotype of hereditary BAP1 cancer predisposition syndrome, reporting three new cases. *Genes, Chromosomes and Cancer*. 2014;53(2):177-182.
19. Thakker RV, Newey PJ, Walls GV, et al. Clinical Practice Guidelines for Multiple Endocrine Neoplasia Type 1 (MEN1). *The Journal of Clinical Endocrinology & Metabolism*. 2012;97(9):2990-3011.
20. Hampel H, Bennett RL, Buchanan A, Pearlman R, Wiesner GL. A practice guideline from the American College of Medical Genetics and Genomics and the National Society of Genetic Counselors: referral indications for cancer predisposition assessment. *Genet Med*. 2015;17(1):70-87.
21. Cancer Facts & Figures 2016.
<http://www.cancer.org/acs/groups/content/@research/documents/document/acspc-047079.pdf>.
22. Schrader KA, Cheng DT, Joseph V, et al. Germline variants in targeted tumor sequencing using matched normal dna. *JAMA Oncology*. 2016;2(1):104-111.
23. Lek M, Karczewski KJ, Minikel EV, et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature*. 2016;536(7616):285-291.
24. Karczewski KJ, Weisburd B, Thomas B, et al. The ExAC browser: displaying reference data information from over 60 000 exomes. *Nucleic Acids Res*. 2017;45(D1):D840-d845.
25. ExAC Browser (Beta) | Exome Aggregation Consortium. [Webpage].
<http://exac.broadinstitute.org/about>. Accessed 2016, August 24.
26. IGSR and the 1000 Genomes Project. IGSR: The International Genome Sample Resource [Webpage]. <http://www.1000genomes.org/>. Accessed August 24, 2016.
27. dbSNP Short Genetic Variations Home Page. [Webpage].
<http://www.ncbi.nlm.nih.gov/projects/SNP/>. Accessed August 24, 2016.
28. IBD Exomes Portal. <http://ibd.broadinstitute.org>. Accessed October 5, 2016.
29. Offit K, Pierce H, Kirchhoff T, et al. Frequency of CHEK2*1100delC in New York breast cancer cases and controls. *BMC medical genetics*. 2003;4:1.