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AMENDED REPORT

Ordered By	Contact ID:6323424	Org ID:8141	
Medical Professional:	Unknown, Unknown, MD		
Client:	MOCKORG44 (10829)		

Patient Legal Name:	**Last, First**		
Accession #:	**01-326965**	Specimen #:	
AP2 Order #:	3105401	Specimen:	Blood EDTA (Purple top)
Birthdate:	01/01/2005	Sex assigned at birth:	F
MRN #:	N/A	Collected:	05/03/2025
Indication:	Diagnostic/Family History	Received:	05/06/2025
		Test Started:	05/06/2025

CancerNext-Expanded®: Analyses of Genes Associated with Hereditary Cancer (90 genes)

AMENDMENT

This report was amended to include new information regarding the *BRCA2* p.F2562L (c.7684T>C) variant, which has been reclassified from "unknown significance" to "likely pathogenic." Updated interpretation information is provided below. This report supersedes all previous reports.

RESULTS

PMS2	**Pathogenic Mutation:**	**c.903G>T**
BRCA2	**Variant, Likely Pathogenic:**	**p.F2562L**

SUMMARY

POSITIVE: Pathogenic Mutation Detected

INTERPRETATION

- This individual is heterozygous for the **c.903G>T (p.K301N)** pathogenic mutation in the *PMS2* gene.
  - This result is consistent with a diagnosis of Lynch syndrome (also known as hereditary non-polyposis colorectal cancer or HNPCC).
  - **Risk estimate**: lifetime risks of 8.7-20% for colorectal cancer and 13-26% for endometrial cancer.
  - Genetic testing for pathogenic mutations in family members can be helpful in identifying at-risk individuals.
- This individual is heterozygous for the **p.F2562L (c.7684T>C)** likely pathogenic variant in the *BRCA2* gene.
  - This result is consistent with a diagnosis of *BRCA2*-related cancer predisposition.
  - **Risk estimate**: **Current risk estimates associated with this variant are not well defined and may be variable, ranging from negligible to high-risk *BRCA2*-related cancer predisposition. Clinical correlation is advised**.
  - Genetic testing for likely pathogenic variants (VLPs) in family members can be helpful in identifying at-risk individuals.
- The expression and severity of disease for this individual cannot be predicted.
- The interactive effect and relative contribution of these alterations on clinical phenotype is unknown at this time.
- Genetic counseling is a recommended option for all individuals undergoing genetic testing.

No additional pathogenic mutations, variants of unknown significance, or gross deletions or duplications were detected. Genes Analyzed (90 total): ***AIP, ALK, APC, ATM, BAP1, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDC73, CDH1, CDK4, CDKN1B, CDKN2A, CEBPA, CHEK2, DICER1, ETV6, FH, FLCN, GATA2, KIF1B, LZTR1, MAX, MEN1, MET, MLH1, MSH2, MSH6, MUTYH, NF1, NF2, NTHL1, PALB2, PHOX2B, PMS2, POT1, PRKAR1A, PTCH1, PTEN, RAD51C, RAD51D, RB1, RET, RPS20, RUNX1, SDHA, SDHAF2, SDHB, SDHC, SDHD, SMAD4, SMARCA4, SMARCB1, SMARCE1, STK11, SUFU, TMEM127, TP53, TSC1, TSC2, VHL*** **and** ***WT1*** **(sequencing and deletion/duplication);** ***ATRIP, AXIN2, CFTR, CPA1, CTNNA1, CTRC, DDX41, EGFR, EGLN1, HOXB13, KIT, MBD4, MITF, MLH3, MSH3, PALLD, PDGFRA, POLD1, POLE, PRSS1, RAD51B, RNF43, SPINK1*** **and** ***TERT*** **(sequencing only);** ***EPCAM*** **and** ***GREM1*** **(deletion/duplication only)**.

***PMS2* Additional Information**

The **c.903G>T** pathogenic mutation (also known as p.K301N), located in coding exon 8 of the *PMS2* gene, results from a G to T substitution at

nucleotide position 903. The lysine at codon 301 is replaced by asparagine, an amino acid with similar properties. However, this change occurs in the last base pair of coding exon 8, which makes it likely to have some effect on normal mRNA splicing. This alteration has been reported in multiple individuals with personal and/or family histories consistent with Lynch syndrome (ten Broeke SW et al. *J. Clin. Oncol.*, 2015 Feb;33:319-25; Goodenberger ML et al. *Genet. Med*., 2016 Jan;18:13-9; Suerink M et al. *Genet. Med*., 2016 Apr;18:405-9; Martin-Morales L et al. *PLoS One*, 2018 Sep;13:e0203885) and several had isolated loss of *PMS2* staining on immunohistochemistry in their Lynch-associated tumors (Senter L et al. *Gastroenterology*. 2008 August;135(2):419-28; Tomsic J et al. *Clin. Genet*. 2013 Mar; 83(3):238-43; van der Klift HM et al. *Mol. Genet. Genomic* Med 2015 Jul; 3(4):327-45). RNA studies have demonstrated that this alteration results in skipping of coding exon 8 (Ambry internal data; van der Klift HM et al. *Mol. Genet. Genomic* Med 2015 Jul; 3(4):327-45). One French individual diagnosed with constitutional mismatch repair deficiency syndrome (CMMRD) was found to carry the c.903G>T mutation in conjunction with another *PMS2* pathogenic mutation (Lavoine N et al. *J. Med. Genet*., 2015 Nov;52:770-8). This nucleotide position is highly conserved in available vertebrate species. This amino acid position is highly conserved in available vertebrate species. *In silico* splice site analysis predicts that this alteration will weaken the native splice donor site. In addition, as a missense substitution this is predicted to be tolerated by *in silico* analysis. Based on the supporting evidence, this alteration is interpreted as a disease-causing mutation.

The *PMS2* gene (NM_000535.5) is located on chromosome 7p22.1, encodes the mismatch repair endonuclease PMS2 protein, and contains 15 coding exons. Pathogenic variants in this gene are known to cause *PMS2*-related Lynch syndrome (also known as hereditary non-polyposis colorectal cancer or HNPCC), which is inherited in an autosomal dominant fashion, and constitutional mismatch repair deficiency (CMMR-D) syndrome, which is inherited in an autosomal recessive fashion. *PMS2*-related Lynch syndrome is characterized by an increased risk for colon cancer (8.7-20% cumulative lifetime risk) and endometrial cancer (13-26% cumulative lifetime risk in females); specifically, the cancer risk is demonstrated to be increased for individuals over the age of 50 (Dominguez-Valentin M et al. *Genet Med*, 2020 01;22:15-25). Risks for cancers of the ovary, small bowel, stomach, pancreas, biliary tract, renal/bladder (urothelial), sebaceous glands (a variant of Lynch syndrome also known as Muir Torre syndrome), or central nervous system may be elevated in individuals with a *PMS2* pathogenic variant compared to the general population (Ten Broeke S et al. *J Clin Oncol*. 2018 Oct 10;36(29):2961-2968; Møller P et al. *Gut,* 2018 07;67:1306-1316; Dominguez-Valentin M et al. *Genet Med*, 2020 01;22:15-25; Wang C et al. *JNCI Cancer Spectr.* 2020 Apr 23;4(5):pkaa027). Some studies have estimated that mutations in mismatch repair (MMR) genes may be related to an approximately 2-fold increase in prostate cancer risk; however, new data suggests that this risk may be attributed primarily to mutations in *MSH2*, and that this risk may be less significant for other MMR genes (Ryan S et al. *Cancer Epidemiol. Biomarkers Prev.* 2014 Mar;23(3):437-49; Dominguez-Valentin 2020). Overall, lifetime colorectal and endometrial cancer risks for individuals with *PMS2* pathogenic variants are estimated to be lower than those associated with pathogenic variants in the *MLH1* and *MSH2* genes (Ten Broeke 2018; Moller 2018, Dominguez-Valentin, 2020). In addition, variable expressivity is observed; therefore, cancer risks will differ based on individual and family history. Loss of function has been reported as the mechanism of disease for *PMS2*-related Lynch syndrome CMMR-D is characterized by café au lait macules and an increased risk for hematologic malignancies, brain tumors, and early-onset Lynch syndrome-associated cancers (Aronson M et al. *J Med Genet*. 2022 Apr;59(4):318-327). Individuals of reproductive age are at 25% risk of having a child with CMMR-D with each pregnancy when both biological parents have a pathogenic variant in *PMS2*. Biallelic loss of function has been reported as the mechanism of disease for CMMR-D.

***BRCA2* Additional Information**

The **p.F2562L** variant (also known as c.7684T>C), located in coding exon 15 of the *BRCA2* gene, results from a T to C substitution at nucleotide position 7684. The phenylalanine at codon 2562 is replaced by leucine, an amino acid with highly similar properties. This alteration co-occurs with a pathogenic *BRCA2* frameshift mutation in a cell line derived from a Fanconi Anemia patient; however, the phase of these two alterations was not described (Stoepker C et al. *DNA Repair (Amst.)*, 2015 Feb;26:54-64). One homology directed repair (HDR) assay found this alteration to be defective (Guidugli L et al. *Am. J. Hum. Genet.*, 2018 Jan), while another HDR assay found this alteration to have intermediate function (Hart SN et al. *Genet Med*, 2019 01;21:71-80). Two saturation genome editing-based studies, including a haploid cell-survival assay and a humanized mouse embryonic stem cell line assay of drug response and survival, demonstrate that this nucleotide substitution may be non-functional (Huang H et al. *Nature*. 2025 Feb;638(8050):528-537; Sahu S et al. *Nature*. 2025 Feb;638(8050):538-545). In a study of 1854 high-risk breast/ovarian cancer families in Italy, this alteration was detected in 1 family (Azzollini J et al. *Eur J Intern Med*, 2016 Jul;32:65-71). This amino acid position is highly conserved in available vertebrate species. In addition, this alteration is predicted to be deleterious by *in silico* analysis. Based on the majority of available evidence to date, this variant is likely to be pathogenic. However, because this variant is identified in one or more patients with Fanconi Anemia it may be hypomorphic, and thus, carriers of this variant and their families may present with reduced risks, and not with the typical clinical characteristics of a high-risk pathogenic *BRCA2* alteration. As risk estimates are unknown at this time, clinical correlation is advised.

The *BRCA2* gene (NM_000059.3) is located on chromosome 13q13.1, encodes the breast cancer type 2 susceptibility protein, and contains 26 coding exons. Pathogenic variants in this gene are known to cause *BRCA2*-related cancer predisposition, which is inherited in an autosomal dominant fashion, and *BRCA2*-related Fanconi anemia, which is inherited in an autosomal recessive fashion. *BRCA2*-related cancer predisposition is characterized by a significantly increased cumulative lifetime risk for female breast cancer (55-69%), male breast cancer (1.8-7.1%), epithelial ovarian cancer (13-29%), pancreatic cancer (5-10%), prostate cancer (19-61%), and melanoma. *BRCA2*-related cancer predisposition is also associated with a contralateral female breast cancer risk of up to 26% within 20 years of initial breast cancer diagnosis with no intervention; however, this risk is age-dependent and more significant with earlier age (prior to age 40) of first breast cancer diagnosis (Kuchenbaecker K et al. *JAMA*. 2017 Jun 20;317(23):2402-2416; Hu C et al. *J Natl Cancer Inst*. 2020 Dec 14;112(12):1231-124; Breast Cancer Association Consortium. *N Engl J Med*. 2021;384:428-439; Hu C et al. *N Engl J Med*. 2021 Feb 4; 384(5): 440–451; Tai Y et al. *J Natl Cancer*

Inst. 2007 Dec 5;99(23):1811-4; Chen J et al. *JNCI Cancer Spectr*. 2020 Apr 23;4(4):pkaa029; Chaffee K et al. *Genet Med*. 2018 Jan;20(1):119-127; Hu C et al. *JAMA*. 2018 Jun 19;319(23):2401-2409). Penetrance in individuals with *BRCA2*-related cancer predisposition is incomplete and variable expressivity is observed; therefore, cancer risks will differ based on individual and family history. Published evidence suggests that both germline and somatic alterations in the *BRCA2* gene predict sensitivity to chemotherapy agents that induce DNA damage and have been included in some indications for approved poly(ADP-ribose) polymerase (PARP) inhibitor therapies (Kim G et al. *Clin Cancer Res*. 2015 Oct 1;21(19):4257-61; Balasubramaniam S et al. *Clin. Cancer Res.*, 2017 Dec;23:7165-7170). Loss of function has been reported as the mechanism of disease for *BRCA2*-related cancer predisposition. *BRCA2*-related Fanconi anemia is characterized by progressive bone marrow failure, adult-onset aplastic anemia, pre- and postnatal growth deficiency, abnormal skin pigmentation, characteristic skeletal malformations, and impaired endocrine functioning. *BRCA2*-related Fanconi anemia can be established in a patient following cytogenetic testing of patient lymphocytes that demonstrate increased chromosomal breakage and radial forms following diepoxybutane and mitomycin C exposure (Mehta P et al. *Fanconi Anemia*. 2002 Feb 14 [updated 2021 Jun 3]. In: *GeneReview*s [Internet]. Seattle (WA): University of Washington, Seattle; 1993–2022). Individuals with *BRCA2*-related Fanconi anemia are at an increased risk of malignancies with highest risk of acute myelogenous leukemia, early-onset solid tumors including head and neck squamous cell carcinoma, and non-melanoma skin cancer (García-de-Teresa B et al. *Genes* (Basel). 2020 Dec 21;11(12):1528, 2020). Individuals of reproductive age are at 25% risk of having a child with Fanconi anemia with each pregnancy when both biological parents have a pathogenic variant in *BRCA2*. Biallelic loss of function, with at least one hypomorphic allele, has been reported as the mechanism of disease for *BRCA2*-related Fanconi anemia.

Order Summary: The following products were included in the test order for this individual. Please note: tests on hold and those that have been cancelled (including reflex testing steps cancelled due to a positive result in a preceding test) are excluded. For additional information, please contact Ambry Genetics.

- CancerNext-Expanded® (Product Code 8875)

ASSAY INFORMATION

General methodology: Genomic deoxyribonucleic acid (gDNA) is isolated from the patient's specimen using standardized methodology and quantified. Sequence enrichment of the targeted coding exons and adjacent intronic nucleotides is carried out by a bait-capture methodology using long biotinylated oligonucleotide probes followed by polymerase chain reaction (PCR) and Next-Generation sequencing (NGS). Variants in regions complicated by pseudogene interference, variant calls not satisfying depth of coverage and variant allele frequency quality thresholds, and potentially homozygous variants are verified by Sanger sequencing. Gross deletion/duplication analysis is performed using a customized pipeline using a combination of third-party coverage-based tools and custom methodologies with confirmatory MLPA and/or targeted chromosomal microarray. Mobile element insertions, if detected, are confirmed by PCR and Sanger sequencing and/or gel electrophoresis.

Additional methodology:

- ***MSH2***: The inversion of coding exons 1-7 is detected by NGS and confirmed by multiplex ligation-dependent probe amplification (MLPA) or PCR and agarose gel electrophoresis.
- ***PMS2****:* Gross deletions and duplications of exons 11-15 of *PMS2* are reflexed to long-range PCR and gel electrophoresis and/or sequencing to determine if the event occurs within *PMS2* or *PMS2CL*. The most likely deletion/duplication configuration that is consistent with the long-range PCR results is reported; however, rare complex rearrangements in *PMS2* and *PMS2CL* cannot be ruled out.

NCBI reference sequences: *AIP*- NM_003977.2, *ALK*- NM_004304.4, *APC*- NM_000038.5 & NM_001127511.2, *ATM*- NM_000051.3, *ATRIP*- NM_130384.1, *AXIN2*- NM_004655.3, *BAP1*- NM_004656.2, *BARD1*- NM_000465.2, *BMPR1A*- NM_004329.2, *BRCA1*- NM_007294.3, *BRCA2*- NM_000059.3, *BRIP1*- NM_032043.2, *CDC73*- NM_024529.4, *CDH1*- NM_004360.3, *CDK4*- NM_000075.3, *CDKN1B*- NM_004064.3, *CDKN2A*- NM_000077.4 & NM_058195.3, *CEBPA*- NM_004364.3, *CFTR*- NM_000492.3, *CHEK2*- NM_007194.3, *CPA1*- NM_001868.2, *CTNNA1*- NM_001903.2, *CTRC*- NM_007272.2, *DDX41*- NM_016222.2, *DICER1*- NM_177438.2, *EGFR*- NM_005228.3, *EGLN1*- NM_022051.2, *EPCAM*- NM_002354.2, *ETV6*- NM_001987.4, *FH*- NM_000143.3, *FLCN*- NM_144997.5, *GATA2*- NM_032638.4, *GREM1*- NM_013372.6, *HOXB13*- NM_006361.5, *KIF1B*- NM_015074.3, *KIT*- NM_000222.2, *LZTR1*- NM_006767.3, *MAX*- NM_002382.3, *MBD4*- NM_001276270.2, *MEN1*- NM_130799.2, *MET*- NM_001127500.1, *MITF*- NM_000248.3, *MLH1*- NM_000249.3, *MLH3*- NM_001040108.1, *MSH2*- NM_000251.1, *MSH3*- NM_002439.3, *MSH6*- NM_000179.2, *MUTYH*- NM_001128425.1, *NF1*- NM_000267.3, *NF2*- NM_000268.3, *NTHL1*- NM_002528.5, *PALB2*- NM_024675.3, *PALLD*- NM_001166110.1, *PDGFRA*- NM_006206.4, *PHOX2B*- NM_003924.3, *PMS2*- NM_000535.5, *POLD1*- NM_002691.2, *POLE*- NM_006231.2, *POT1*- NM_015450.2, *PRKAR1A*- NM_002734.3, *PRSS1*- NM_002769.4, *PTCH1*- NM_000264.3, *PTEN*- NM_000314.4, *RAD51B* - NM_133510.3, *RAD51C*- NM_058216.1, *RAD51D*- NM_002878.3, *RB1*- NM_000321.2, *RET*- NM_020975.4, *RNF43*- NM_017763.4, *RPS20*- NM_001023.3, *RUNX1*- NM_001754.4, *SDHA*- NM_004168.2, *SDHAF2*- NM_017841.2, *SDHB*- NM_003000.2, *SDHC*- NM_003001.3, *SDHD*- NM_003002.2, *SMAD4*- NM_005359.5, *SMARCA4*- NM_001128849.1, *SMARCB1*- NM_003073.3, *SMARCE1*- NM_003079.4, *SPINK1*- NM_003122.3, *STK11*- NM_000455.4, *SUFU*- NM_016169.3, *TERT* - NM_198253.2, *TMEM127*- NM_017849.3, *TP53*- NM_000546.4, *TSC1*- NM_000368.4, *TSC2*- NM_000548.3, *VHL*- NM_000551.3, and *WT1*- NM_024426.4.

Analytical range: This test detects variants in the coding domains and well into the flanking 5' and 3' ends of the introns and untranslated regions. Unless explicitly stated, sequence and copy number variants in the promoter, non-coding exons, or 3' untranslated regions are not routinely reported.

Analytical range exceptions:

- ***APC***: all promoter 1B gross deletions as well as single nucleotide substitutions within the promoter 1B YY1 binding motif (NM_001127511 c.-196_-186) are analyzed and reported.
- ***EPCAM***: only gross deletions encompassing the 3' end of the gene are reported.
- ***GREM1***: only the status of the 40kb 5'UTR gross duplication is analyzed and reported.
- ***MITF***: only the c.952G>A (p.E318K) variant is reported.
- ***MSH3*** and ***PHOX2B***: the polyalanine repeat regions are excluded from analysis.
- ***NTHL1***: only full-gene gross deletions and duplications are detected.
- **Gross deletion/duplication analysis is not performed for the following genes:** *ATRIP,AXIN2, CFTR, CPA1, CTNNA1, CTRC, DDX41, EGFR, EGLN1, HOXB13, KIT, MBD4, MITF, MLH3, MSH3, PALLD, PDGFRA, POLD1, POLE, PRSS1, RAD51B, RNF43, SPINK1*, and *TERT*.

Reporting: Results reported herein may be of constitutional or somatic origin. This methodology cannot differentiate between these possibilities. In result reports, variants in the following classifications are always reported, and are based on the following definitions and clinical recommendations.

- **Pathogenic Variant**: variants with sufficient evidence to classify as pathogenic (capable of causing disease). Targeted testing of at-risk relatives and appropriate changes in medical management for pathogenic variant carriers recommended. Previously described pathogenic variants, including intronic variants at any position, are always reported when detected.

- **Variant, Likely Pathogenic (VLP)**: variants with strong evidence in favor of pathogenicity. Targeted testing of at-risk relatives and appropriate changes in medical management for VLP carriers typically recommended. Previously described likely pathogenic variants, including intronic VLPs at any position, are always reported when detected.
- **Variant, Uncertain Significance (VUS)**: variants with limited and/or conflicting evidence regarding pathogenicity. Familial testing via the Family Studies Program may be recommended. Medical management to be based on personal/family clinical histories, not VUS carrier status. Note, intronic VUSs are always reported out to 5 base pairs from the splice junction when detected.

Variants of unlikely clinical significance (those with strong/very strong evidence to argue against pathogenicity) are not routinely included in results. These include findings classified as “likely benign” and “benign” variants. Classification and interpretation of variants may change over time with accumulating evidence and scientific advancements. Updated classifications may be reported through reclassification notices; however, clients should re-contact the laboratory or visit ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) for the most up to date information regarding the current interpretation of results.

All results, including those from prior genetic testing for themselves and/or family members, will be reported as described above.

Gender identity (if provided) is not used in the interpretation of results, and sex assigned at birth is used in the interpretation of results only when necessary. Currently, there are insufficient data to determine specific cancer risk adjustments for transgender, nonbinary, or intersex individuals.

Assay Information Continued on Next Page

ASSAY INFORMATION *(Supplement to Test Results - Continued)*

Resources: The following references are used in variant analysis and classification when applicable for observed genetic alterations.

1. The 1000 Genomes Project Consortium. An integrated map of genetic variation from 1092 human genomes. *Nature*. 2012;491:56-65.
2. ACMG Standards and guidelines for the interpretation of sequence variants. *Genet Med.* 2015 May;17(5):405-23.
3. Ambry Genetics Variant Classification Scheme. http://www.ambrygen.com/variant-classification.
4. Berkeley Drosophila Genome Project [Internet]. Reese MG et al. *J Comp Biol.* 1997;4:311-23. http://www.fruitfly.org/seq_tools/splice.html.
5. Database of Single Nucleotide Polymorphisms (dbSNP) [Internet]. Bethesda (MD): National Center for Biotechnology Information, National Library of Medicine (dbSNP Build ID:135) Available from: www.ncbi.nlm.nih.gov/SNP. Accessed Jan 2012).
6. ESEfinder [Internet]. Smith PJ, et al. (2006) *Hum Mol Genet.* 15(16):2490-2508 and Cartegni L, et al. *Nucleic Acid Research.* 2003;31(13):3568-3571. http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi?process=home.
7. Exome Variant Server, NHLBI Exome Sequencing Project (ESP) [Internet], Seattle WA. Available from: evs.gs.washington.edu/EVS.
8. Grantham R. Amino acid difference formula to help explain protein evolution. *Science.* 1974;185(4151):862-864.
9. HGMD® [Internet]: Stenson PD et al. *Genome Med.* 2009;1(1):13. www.hgmd.cf.ac.uk.
10. Landrum MJ et al. ClinVar: public archive of relationships among sequence variation and human phenotype. *Nucleic Acids Res*. 2014 Jan 1;42(1):D980-5. doi: 10.1093/nar/gkt1113. PubMed PMID: 24234437.
11. Online Mendelian Inheritance in Man, OMIM®. McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, MD), Copyright® 1966-2012. World Wide Web URL: http://omim.org.
12. Feng BJ. PERCH: A Unified Framework for Disease Gene Prioritization. *Hum Mutat.* 2017 Mar;38(3):243-251.
13. Exome Aggregation Consortium (ExAC) [Internet], Cambridge, MA. Available from: http://exac.broadinstitute.org.
14. Genome Aggregation Database (gnomAD) [Internet], Cambridge, MA. Available from: http://gnomad.broadinstitute.org.
15. Lek M et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature*. 2016 Aug 17;536(7616):285-91. PMID: 27535533
16. Mu W et al. *J Mol Diagn*. 2016 Oct 4. PubMed PMID: 27720647
17. Karczewski KJ et al. *Nature*. 2020 May;581(7809):434-443. PMID: 32461654
18. Splicing Prediction: Jaganathan K et al. *Cell.* 2019 Jan 24; 176(3):535-548.e24. PMID: 30661751

Disclaimer: This test was developed, and its performance characteristics were determined by Ambry Genetics Corporation. It has not been cleared or approved by the US Food and Drug Administration (FDA). The FDA does not require this test to go through premarket FDA review. It should not be regarded as investigational or for research. This test should be interpreted in context with other clinical findings. This report does not represent medical advice. Any questions, suggestions, or concerns regarding interpretation of results should be forwarded to a genetic counselor, medical geneticist, or physician skilled in interpretation of the relevant medical literature. This laboratory is certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity clinical laboratory testing. This test analyzes the following types of mutations: nucleotide substitutions, small deletions (up to 25 bp), small insertions (up to 10 bp), small indels, and gross deletions/duplications. Unless otherwise noted in the methodology section above, this test is not intended to analyze the following types of alterations: gross rearrangements, deep intronic variations, mobile element insertions, and other unknown abnormalities. The pattern of mutation types varies by gene, and this test detects a high but variable percentage of known and unknown mutations of the classes stated. A negative result from the analysis cannot rule out the possibility that the tested individual carries a rare unexamined mutation or mutation in the undetectable group. This test is designed and validated to be capable of detecting ~99.9% of described mutations in the genes represented on the test, listed above (analytical sensitivity). The clinical sensitivity of this test may vary widely according to the specific clinical and family history. Mutations in other genes or the regions not analyzed by this test can also give rise to similar clinical conditions. Although molecular tests are highly accurate, rare diagnostic errors may occur. Possible diagnostic errors include sample mix-up, erroneous paternity identification, technical errors, clerical errors, and genotyping errors. Genotyping errors can result from trace contamination of PCR reactions, from maternal cell contamination in fetal samples, from rare genetic variants that interfere with analysis, germline or somatic mosaicism, presence of pseudogenes, technical difficulties in regions with high GC content or homopolymer tracts, active hematologic disease, a history of allogeneic bone marrow or peripheral stem cell transplant, or from other sources. Rare variants present in the human genome reference sequence (GRCh37.p5/hg19) or rare misalignment due to presence of pseudogenes can lead to misinterpretation of patient sequence data.

Ambry Genetics®

Clinician Management Resource for *PMS2* (Lynch syndrome)

This overview of clinical management guidelines is based on this patient's positive test result for a pathogenic or likely pathogenic variant in the *PMS2* gene. Unless otherwise stated, medical management guidelines used here are limited to those issued by the National Comprehensive Cancer Network® (NCCN®)[1] in the U.S. Please consult the referenced guideline for complete details and further information.

Clinical correlation with the patient's past medical history, treatments, surgeries and family history may lead to changes in clinical management decisions; therefore, other management recommendations may be considered. Genetic testing results and medical society guidelines help inform medical management decisions but do not constitute formal recommendations. Discussions of medical management decisions and individualized treatment plans should be made in consultation between each patient and his or her healthcare provider, and may change over time.

SCREENING/SURGICAL CONSIDERATIONS[1]	AGE TO START	FREQUENCY
Colorectal Cancer		
Colonoscopy	30-35 years old (or 2-5 years prior to the earliest colorectal cancer in the family, if it is diagnosed before 30 years)	Every 1-3 years^
Consider daily aspirin to reduce future risk of colorectal cancer, including a discussion of risks and benefits. Patients with *PMS2*-associated Lynch syndrome may be less likely to experience significant benefit.	Individualized	N/A
Endometrial (Uterine) Cancer		
Encourage prompt response to symptoms (e.g. abnormal uterine bleeding, postmenopausal bleeding).	Individualized	Individualized
Consider screening via endometrial biopsy. Routine endometrial cancer screening does not have proven benefit.	30-35 years old	Every 1-2 years
Consider the option of risk-reducing hysterectomy.	Hysterectomy with bilateral salpingo-oophorectomy: starting at 50 years old	N/A
Transvaginal ultrasound may be considered in post menopausal patients.^^	Individualized	Individualized
Consider risk-reduction agents, including oral contraceptive pills and progestin intrauterine systems.	Individualized	Individualized
Ovarian Cancer		
Insufficient evidence exists to make a specific recommendation for risk-reducing salpingo-oophorectomy for *PMS2* pathogenic variant carriers. Patients with pathogenic variants in *PMS2* appear to be at no greater than average risk for ovarian cancer, and may consider deferring surveillance and may reasonably elect not to have oophorectomy. As premature menopause due to oophorectomy can cause detriments to bone health, cardiovascular health, and generalized quality of life, estrogen replacement therapy should be considered for patients who elect to have a BSO. Salpingectomy is also an option for premenopausal patients who are not yet ready for oophorectomy.	Hysterectomy with bilateral salpingo-oophorectomy: starting at 50 years old	N/A
CA-125 and pelvic ultrasound are recommended for preoperative planning. Data do not support routine ovarian screening.	Individualized	Individualized
Consider risk-reduction agents, including oral contraceptive pills and progestin intrauterine systems.	Individualized	Individualized
Urothelial Cancer		
Selected individuals such as those with a family history of urothelial cancer may consider urinalysis. There is insufficient evidence to recommend a particular surveillance strategy.	30-35 years old	Every 12 months

SCREENING/SURGICAL CONSIDERATIONS[1]	AGE TO START	FREQUENCY
Gastric and Small Bowel Cancer		
Consider upper GI surveillance with high-quality endoscopic gastroduodenoscopy, preferably in conjunction with colonoscopy. Random biopsy of the proximal and distal stomach should at a minimum be performed on the initial procedure to assess for *H. pylori*, autoimmune gastritis, and intestinal metaplasia.	30-40 years old or earlier based on family history or high risk findings	Every 2-4 years or more frequently based on family history or high-risk findings
Individuals not undergoing endoscopic surveillance should have one-time noninvasive testing for *H. pylori* at time of Lynch syndrome diagnosis.	Individualized	N/A
Treatment for *H. pylori* if detected.	Individualized	N/A
Pancreatic Cancer		
While *PMS2* carriers have not been shown to have an increased risk for pancreatic tumors, pancreatic screening may still be considered for individuals with exocrine pancreatic cancer in >1 first-or second-degree relative on the same side of the family as the identified pathogenic/likely pathogenic germline variant.*	Individualized	Individualized
Prostate Cancer		
It is reasonable for men with Lynch syndrome to consider beginning shared decision-making about prostate cancer screening.	40 years old	Consider screening at annual intervals rather than every other year.
Breast Cancer		
Not enough evidence to support increased screening above average-risk screening recommendations or based on personal and/or family history.	Individualized	Individualized
Brain Cancer		
Consider physical/neurological examination	Patients should be educated regarding signs and symptoms of neurologic cancer and the importance of prompt reporting of abnormal symptoms to their physicians.	
Skin Manifestations		
Consider skin exam with a health care provider skilled in identifying Lynch syndrome-associated skin manifestations.	Individualized	Every 1-2 years
Reproductive Options		
For patients of reproductive age, counsel about options for prenatal diagnosis and assisted reproduction, including pre-implantation genetic testing.	Individualized	N/A
If both parents are carriers of a pathogenic/likely pathogenic variant in *PMS2*, counsel for risk of a rare autosomal recessive condition called constitutional mismatch repair deficiency (CMMRD).	Individualized	N/A
Risk to Relatives		
Advise patients to tell their relatives about possible inherited cancer risk, options for risk assessment, and management. Recommend genetic counseling and consideration of genetic testing for at-risk relatives.	Individualized	N/A

^ Individuals who may benefit from a shorter screening interval (ie, 1-year vs 2-year) include those with risk factors such as a history of colorectal cancer or adenoma, male sex, and age over 40 years.

^^ Transvaginal ultrasound is not highly sensitive or specific for endometrial cancer screening.

* For individuals considering pancreatic cancer screening, the guideline recommends that screening be performed in experienced high-volume centers. The guideline recommends that such screening only take place after an in-depth discussion about the potential limitations to screening, including cost, the high incidence of benign or intermediate pancreatic abnormalities, and uncertainties about the potential benefits of pancreatic cancer screening. The guideline recommends that screening be considered using annual contrast-enhanced MRI/MRCP and/or EUS, with consideration of shorter screening intervals for individuals found to have worrisome abnormalities on screening. The guideline emphasizes that most small cystic lesions found on screening will not warrant biopsy, surgical resection, or any other intervention.

1. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Genetic/Familial High-Risk Assessment: Colorectal, Endometrial, and Gastric. v3.2024. Accessed October 31, 2024. To view the most recent and complete version of the guideline, go online to NCCN.org. NCCN makes no warranties of any kind whatsoever regarding their content, use or application and disclaims any responsibility for their application or use in any way.

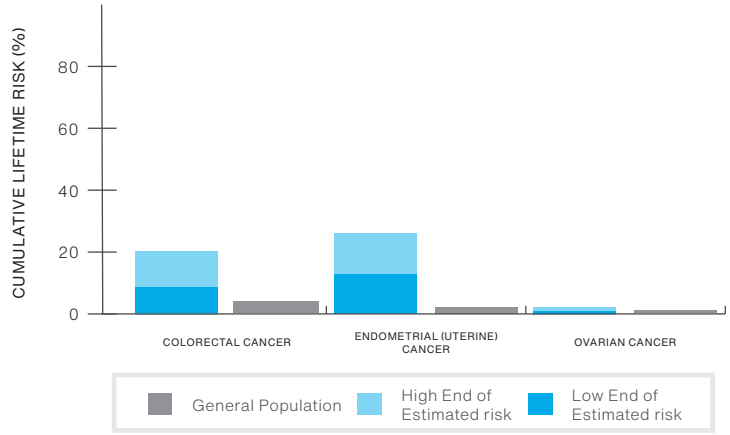
Understanding Your Positive *PMS2* Genetic Test Result

INFORMATION FOR PATIENTS WITH A PATHOGENIC OR LIKELY PATHOGENIC VARIANT

6 Things To Know

1	Result	Your testing shows that you have a pathogenic or likely pathogenic variant in the *PMS2* gene.
2	Lynch syndrome	People with pathogenic or likely pathogenic *PMS2* variants have Lynch syndrome, previously known as hereditary non-polyposis colorectal cancer (HNPCC).
3	Cancer risks	You have an increased chance to develop colorectal, endometrial/uterine, stomach, ovarian, small bowel, and other types of cancer.
4	What you can do	Risk management decisions are very personal. There are options to detect cancer early or lower the risk to develop cancer. It is important to discuss these options with your healthcare provider and decide on a plan that works for you.
5	Other Medical Concerns	Individuals with pathogenic or likely pathogenic *PMS2* variants may have an increased risk to have a child with constitutional mismatch repair deficiency (CMMRD), but only if their partner also carries a pathogenic or likely pathogenic variant in the *PMS2* gene. CMMRD is a multisystem disorder characterized by specific physical features and an increased risk for hematologic malignancies, brain tumors, and early-onset Lynch syndrome-associated cancers.
6	Family	Family members may also be at risk – they can be tested for the pathogenic or likely pathogenic *PMS2* variant that was identified in you. It is recommended that you share this information with family members so they can learn more and discuss this with their healthcare providers.

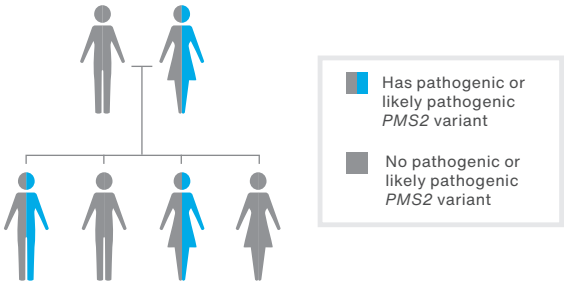
PMS2 Lifetime Cancer Risks*



* Because risk estimates vary in different studies, only approximate risks are given. Cancer risks will differ based on individual and family history.

PMS2 in the Family

There is a 50/50 random chance to pass on the pathogenic or likely pathogenic *PMS2* variant to each of your children.



RESOURCES

- AliveAndKickn (Patient Advocacy Group) aliveandkickn.org
- Lynch Syndrome International lynchcancers.com
- National Society of Genetic Counselors nsgc.org
- Canadian Association of Genetic Counsellors cagc-accg.ca

Please discuss this information with your healthcare provider. The cancer genetics field is continuously evolving, so updates related to your *PMS2* result, medical recommendations, and/or potential treatments may be available over time. This information is not meant to replace a discussion with a healthcare provider, and should not be considered or interpreted as medical advice.

One Enterprise, Aliso Viejo, CA 92656 USA Toll Free +1.866.262.7943 Fax +1.949.900.5501 **ambrygen.com**

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PROMPT

Prospective Registry Of MultiPlex Testing

Opportunity to Enroll in Hereditary Cancer Research

Genetic testing can help individuals and families by giving them a clearer idea of their cancer risks. Genetic tests (called multi-gene or multiplex panels) look for changes in several different genes, all in a single test. While all of the genes on these panels have been tied to an increased risk of cancer, we understand the risks associated with some of the genes better than we understand others. One way to help improve our understanding is to enroll people with pathogenic mutations or variants of unknown significance in registries. Registries typically follow people over many years to learn more about these alterations and how they impact their health.

How can I find a research registry?

There are several hereditary cancer research registries that are studying individuals who have had multiplex panel testing. One registry that is open to individuals nationwide is PROMPT (or **P**rospective **R**egistry **O**f **M**ulti**P**lex **T**esting). PROMPT is an online registry for patients and families who have had multiplex testing and have been found to have a genetic variation which may be linked to an increased risk of cancer. PROMPT is a joint effort involving several academic medical centers and commercial laboratories, working together to learn more about the genes that are studied on multiplex panels. PROMPT will allow researchers to better understand the cancer risks associated with changes in these genes and thus provide a better understanding of the best way to take care of individuals who have such changes.

What is involved in participation?

Participation in the study simply involves completing online surveys. Additionally, the PROMPT team may reach out to you to talk about ways that you can get more involved with the research effort. Your participation will help researchers learn more and improve the ability of this genetic testing to help people.

How do I enroll?

You can learn more about or register for PROMPT by going to www.promptstudy.info or by scanning the QR code below.

Thank you again for considering taking part in PROMPT!

If you would like to read more about multiplex panels, including details about specific genes, please visit our informational website at www.promptstudy.info.

Opportunity to connect and help prevent cancer in your family

Did you recently have genetic testing for a cancer gene variant (or mutation) known to be in your family? Questions such as “Where did this variant come from?” or “What can I do to help others in my family?” are common. ConnectMyVariant can help!

ConnectMyVariant provides resources for people who want help talking with relatives about cancer risk or finding new relatives who might be at risk to help them get genetic testing and prevent cancer.

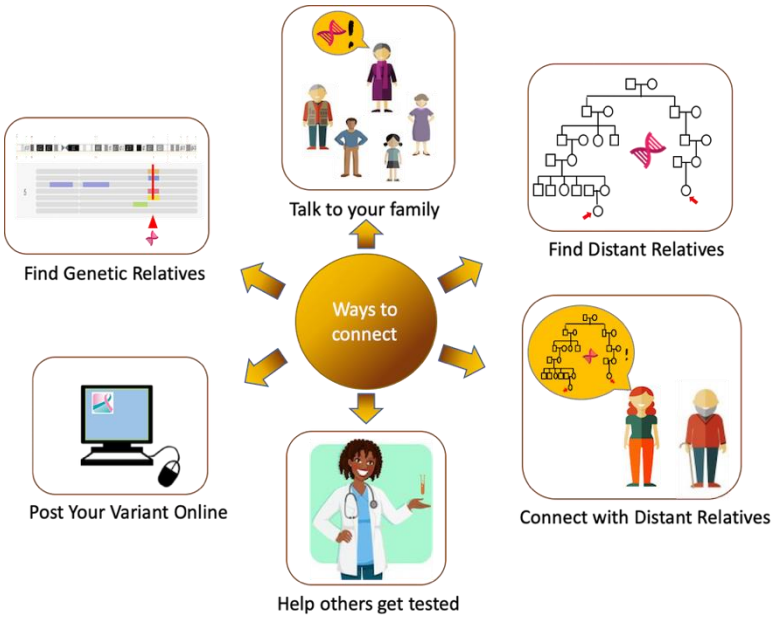
The ConnectMyVariant initiative seeks to help people like you:

- Talk to their relatives
- Share important genetic information
- Expand family trees to identify and connect with distant at-risk relatives
- Guide at-risk relatives to cancer prevention.

“Prevention Through Connection”

People with the same genetic variant may be distantly related through a long-ago ancestor. This means that your family’s variant may be a key to understanding your family’s past. It is also a key that you can use to help both close and distant family members prevent cancer before it happens.

You may have received genetic testing because someone cared enough to warn you about your risk. Now you can find and warn other at-risk relatives. Reaching out and speaking to other at-risk relatives to help them get genetic testing may help prevent cancer and save lives. These are the goals of ConnectMyVariant.



You can learn more and sign up at http://connectmyvariant.org/
Questions? info@connectmyvariant.org

ICARE
INHERITED CANCER REGISTRY

WHY PARTICIPATE IN ICARE?

Be a part of new discoveries.

Studies that used information from ICARE participants have...

found that removing the ovaries may not lower breast cancer risk for women with a ***BRCA*** mutation.[1]

improved cancer risk estimates for people with ***PALB2*** mutations.[2]

Get care updates personalized to you

as new guidelines and other information come out – for example:

ICARE participants with mutations in ***PALB2***, ***CHEK2***, and ***ATM*** were given updates that might affect their care because new National Comprehensive Cancer Network (NCCN) Genetics Guidelines were released in September 2022.

Find out about other studies.

Examples of studies include:

A study providing free resources to help with managing cancer risks and family communication of test results.

A study doing free genomic studies on breast cancers in people with ***BRCA1***, ***BRCA2***, ***PALB2***, ***ATM***, and ***CHEK2*** mutations to learn more about how these tumors develop and how we might best treat them.

Enroll online by visiting
https://redcap.link/ICAREconsent
or **scan the below QR code**:

[1] *Kotsopoulos J, et al. Bilateral Oophorectomy and the Risk of Breast Cancer in BRCA1 Mutation Carriers: A Reappraisal. Cancer Epidemiol Biomarkers Prev. 2022 Jul 1;31(7):1351-1358. PMID: 35477169;* [2] *Yang X, et al. Cancer Risks Associated With Germline PALB2 Pathogenic Variants: An International Study of 524 Families. J Clin Oncol. 2020 Mar 1;38(7):674-685. PMID: 31841383.*

WHAT ARE PARTICIPANTS SAYING ABOUT ICARE?

Participant Testimonials:

"I absolutely love being a part of ICARE... and enjoy receiving their periodic newsletters on clinical and research updates."

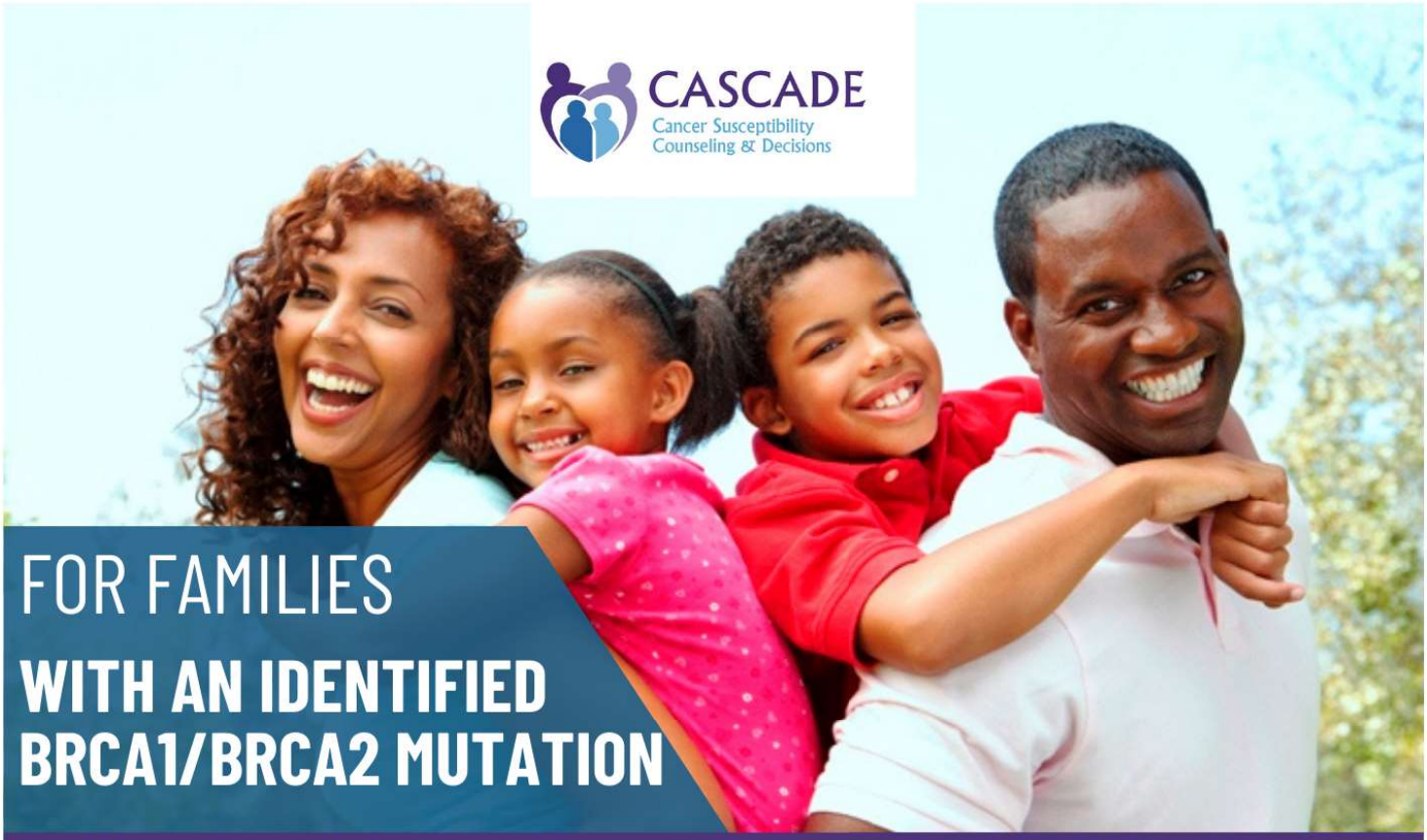
"As much as it might seem frightening to some to join a registry like this, I am grateful for the opportunity to help pay it forward by supporting inherited cancer studies in the hopes we can all live well and have long healthy lives."

"I participate in ICARE and other related activities in hopes that continued research will positively impact all of us with hereditary cancers, and especially my three children who are now young adults."

615-875-2444 ICARE@vumc.org InheritedCancer.net

Version 10/28/2022

CASCADE
Cancer Susceptibility Counseling & Decisions



FOR FAMILIES
WITH AN IDENTIFIED BRCA1/BRCA2 MUTATION

We evaluate the impact of free, expedited web-based genetic education and optional streamlined testing

About CASCADE

Communicating with relatives about your BRCA mutation is key to ensuring that your family gets the information they need to make good decisions about their health. The "Cancer Susceptibility Counseling and Decisions" (CASCADE) study is a National Cancer Institute funded clinical study testing individualized web-based genetic education and optional genetic testing for your adult relatives.

CASCADE and You

- Our goal is to help individuals who are at risk of having a BRCA1/2 mutation. To do this, we need your help. We are asking you to put us in touch with your untested relatives so that we can send them print information about CASCADE.
- If your relatives choose to participate, they will be given access to either: an individually-tailored genetic education website or standard resources about BRCA1/2 testing. These resources will provide them with important cancer risk information.
- Even if your relatives are not interested in genetic information, we would still like them to complete our survey so we can understand why they are not interested.
- All participants will have the option to pursue genetic testing if they so choose. All information gathered during this study will remain completely confidential.
- To get started, go to **https://redcap.link/CASCADE** and complete our breif screener to confirm your eligibility.

The Jess and Mildred Fisher Center for Hereditary Cancer and Clinical Genomics Research

Georgetown University | Georgetown Lombardi Comprehensive Cancer Center
2115 Wisconsin Avenue, NW | Suite 300 | Washington, DC 20007
email: cascadestudy@georgetown.edu

NCI Comprehensive Cancer Center
A Cancer Center designated by the National Cancer Institute