VOLUME 36 · NUMBER 35 · DECEMBER 10, 2018

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Identification of Incidental Germline Mutations in Patients With Advanced Solid Tumors Who Underwent Cell-Free Circulating Tumor DNA Sequencing

Thomas P. Slavin, Kimberly C. Banks, Darya Chudova, Geoffrey R. Oxnard, Justin I. Odegaard, Rebecca J. Nagy, Kar Wing Kevin Tsang, Susan L. Neuhausen, Stacy W. Gray, Massimo Cristofanilli, Angel A. Rodriguez, Aditya Bardia, Brian Leyland-Jones, Mike F. Janicek, Michael Lilly, Guru Sonpavde, Christine E. Lee, Richard B. Lanman, Funda Meric-Bernstam, Razelle Kurzrock, and Jeffrey N. Weitzel

Author affiliations and support information (if applicable) appear at the end of this article.

Published at jco.org on October 19, 2018.

T.P.S. and K.C.B. contributed equally to this work.

Corresponding author: Thomas P. Slavin, MD, City of Hope National Medical Center, Bldg 173, Room 131, 1500 E Duarte Rd, Duarte, CA 91010; Twitter: @cityofhope; e-mail: tslavin@coh.org.

0732-183X/18/3635w-3459w/$20.00

ABSTRACT

Purpose
To determine the potential for detection of incidental germline cancer predisposition mutations through cell-free DNA (cfDNA) analyses in patients who underwent solid tumor somatic mutation evaluation.

Patients and Methods
Data were evaluated from 10,888 unselected patients with advanced (stage III/IV) cancer who underwent Guardant360 testing between November 2015 and December 2016. The main outcome was prevalence of putative germline mutations identified among 16 actionable hereditary cancer predisposition genes.

Results
More than 50 cancer types were studied, including lung (41%), breast (19%), colorectal (8%), prostate (6%), pancreatic (3%), and ovarian (2%). Average patient age was 63.5 years (range, 18 to 95 years); 43% were male. One hundred and fifty-six individuals (1.4%) had suspected hereditary cancer mutations in 11 genes. Putative germline mutations were more frequent in individuals younger than 50 years versus those 50 years and older (3.0% *v* 1.2%, respectively; $P < .001$). Highest yields of putative germline findings were in patients with ovarian (8.13%), prostate (3.46%), pancreatic (3.34%), and breast (2.2%) cancer. Putative germline mutation identification was consistent among 12 individuals with multiple samples. Patients with circulating tumor DNA copy number variation and/or reversion mutations suggestive of functional loss of the wild-type allele in the tumor DNA also are described.

Conclusion
Detection of putative germline mutations from cfDNA is feasible across multiple genes and cancer types without prior mutation knowledge. Many mutations were found in cancers without clear guidelines for hereditary cancer genetic counseling/testing. Given the clinical significance of identifying hereditary cancer predisposition for patients and their families as well as targetable germline alterations such as in *BRCA1* or *BRCA2*, research on the best way to validate and return potential germline results from cfDNA analysis to clinicians and patients is needed.

J Clin Oncol 36:3459-3465. © 2018 by American Society of Clinical Oncology

INTRODUCTION

Identification of individuals and families at high risk for cancer can lead to opportunities for cancer prevention and early detection and can influence cancer treatment and management decisions.[1-3] However, current guideline-directed genetic testing for cancer risk misses a significant portion of hereditary cancer predisposition gene mutation carriers.[4,5]

Germline mutation detection in the context of tumor genomic sequencing has been reported in single-institution and retrospective consortia (eg, The Cancer Genome Atlas) settings,[6,7] although established mechanisms for return of results are lacking. Novel methods to identify patients with germline mutations could serve as useful adjuncts to conventional genetic cancer risk assessment.[2,3] In addition, mutations in germline homologous recombination repair genes (eg, *BRCA1*, *BRCA2*) identify candidates for

ASSOCIATED CONTENT

Appendix
DOI: https://doi.org/10.1200/JCO.18.00328

DOI: https://doi.org/10.1200/JCO.18.00328

poly (ADP-ribose) polymerase inhibitor treatment and other targeted therapies in clinical trials.[8]

Cell-free DNA (cfDNA) studies in cancer have shown promise for tumor detection, molecular heterogeneity assessments, identification of therapeutically relevant genomic alterations, and monitoring of tumor dynamics and response to therapy.[9] Incidental maternal germline cancer risk gene mutations have been reported in noninvasive prenatal cfDNA testing for fetal aneuploidies.[10] Similarly, because circulating tumor cfDNA (ctDNA) sequencing is from mostly leukocyte-derived DNA, cfDNA next-generation sequencing (NGS) for somatic tumor mutations may identify heterozygous germline mutations at approximately 50% mutant allele fraction (MAF), which generally are distinguishable from somatic mutations that typically occur at lower MAFs.[11]

Few studies have evaluated germline cancer predisposition through cfDNA,[12-14] and no publications have reported the utility of ctDNA analyses in hereditary predisposition mutation detection across multiple genes or cancer types when the germline mutation status is unknown. The purpose of this study was to describe putative (ie, probable) incidental germline mutations identified through commercial cfDNA testing of patients who underwent solid tumor somatic genotyping. Furthermore, we define clinical factors associated with putative germline mutation detection.

PATIENTS AND METHODS

Participants and Sequencing

This observational, noninterventional case series (completed under Quorum Review Institutional Review Board protocol 30-001) included coded data from 11,681 de-identified cfDNA samples from 10,888 consecutive patients with advanced (all stage III/IV) solid tumors who underwent Guardant360 (Guardant Health, Redwood City, CA) testing (70 to 73 genes) as part of clinical care between November 2015 and December 2016. No individuals with sequencing data available were excluded from the analysis. cfDNA was extracted from plasma, quantified, and used to prepare sequencing libraries, which were sequenced to approximately 15,000 times the average read depth using methods previously described.[15,16] The Guardant360 assay detects single nucleotide variants, small (fewer than 50 bases) indels, *MET* exon 14 large indels, copy number amplifications, and fusion events.

Variant Curation

Variants in 16 clinically actionable genes with defined hereditary cancer associations were analyzed, including *APC*, *ATM*, *BRCA1*, *BRCA2*, *CDKN2A*, *KIT*, *MLH1*, *NF1*, *PTEN*, *RB1*, *RET*, *SMAD4*, *STK11*, *TP53*, *TSC1*, and *VHL* (Table 1). Of this list, full exon sequencing was performed for *BRCA1*, *BRCA2*, *CDKN2A*, *KIT*, *NF1*, *PTEN*, *RB1*, *TP53*, and *VHL*; select exons (those with previously reported somatic mutations) were sequenced for the remaining genes.

All variants suspected to be of germline origin (allele fraction, 40% to 60%) were included in the screen for pathogenic germline alterations. The allele fraction cutoff was selected as a conservative approach on the basis of expected 50% MAFs with a standard deviation of 2.3% for germline heterozygous alterations. Our decision rule to evaluate variants of 40% or more allele fraction was selected a priori without additional adjustments. Variants with an allele fraction less than 40% are more likely to represent somatic alterations related to ctDNA or clonal hematopoiesis.[17,18] In addition, variants with allele fractions greater than 60% were included if a concomitant copy number change was identified, concordant with deviations in allele fractions for neighboring common germline single nucleotide polymorphisms (SNPs) from their expected allele fractions. Generally, such deviations may occur as a result of either copy number gain of the variant allele or copy number loss of the wild-type (nonvariant containing) allele. Equivocal cases with high ctDNA load (defined as more than one circulating tumor mutation present at an MAF greater than 30%), were manually reviewed for relevant gene copy number status and allele frequencies of common neighboring germline SNPs to characterize

Table 1. Putative Germline Mutation Occurrences by Cancer Type

Patient Characteristic	Cancer Type, No. (%)							
	All	Ovarian	Prostate	Pancreatic	Breast	Lung	Colorectal*	Other†
No. of patients	10,888	210	617	332	2,064	4,459	878	2,328
Sex								
Female	6,242 (57.3)	210 (100)	0 (0)	143 (43.1)	2,037 (98.7)	2,449 (54.9)	397 (45.2)	1,006 (43.2)
Male	4,646 (42.7)	0 (0)	617 (100)	189 (56.9)	27 (1.3)	2010 (45.1)	481 (54.8)	1,322 (56.8)
Mean age, years (range)	63.6 (18-95)	62.3 (25-89)	68.7 (35-89)	64.5 (34-89)	58.6 (25-95)	66.4 (20-89)	59.5 (21-89)	62.4 (18-89)
Patients with putative germline mutation	156 (1.4)	17 (8.1)	21 (3.4)	11 (3.3)	45 (2.2)	33 (0.7)	5 (0.6)	24 (1)
Gene								
BRCA2	81	3	20	7	27	17	1	6
BRCA1	41	14		1	10	11	1	4
CDKN2A	10			1	3	2		4
ATM	5			1	2	2		
TP53	5				3			2
APC	4						1	3
NF1	4		1			1		2
RB1	2							2
RET	2			1				1
MLH1	1						1	
SMAD4	1						1	

NOTE. No putative germline mutations were identified in *PTEN*, *STK11*, *TSC1*, *KIT*, or *VHL*. Full coding sequence data were available for *BRCA1*, *BRCA2*, *CDKN2A*, *KIT*, *NF1*, *PTEN*, *RB1*, *TP53*, and *VHL*.

*Lynch syndrome genes not sequenced except for *MLH1* exon 12.

†Other types include carcinoma of unknown primary, endometrial carcinoma, melanoma, thyroid carcinoma, gastric adenocarcinoma, cholangiocarcinoma, renal pelvic urothelial carcinoma, renal cell carcinoma, neuroendocrine carcinoma, and sarcoma.

variants as germline or somatic. If manual review did not adjudicate status, the equivocal variant was not included in the putative germline set.

Using Ingenuity Variant Analysis (QIAGEN, Redwood City, CA), variants observed with an allele frequency of 2.0% or greater in the 1000 Genomes Project,[19] National Heart, Lung, and Blood Institute Exome Sequencing Project,[20] Exome Aggregation Consortium,[21] or Genome Aggregation Database[21] databases were excluded. Remaining single nucleotide variants and indels suspicious for germline origin were classified by Ingenuity Variant Analysis as pathogenic, likely pathogenic, unknown significance, likely benign, or benign according to American College of Medical Genetics and Genomics guidelines.[22] All pathogenic and likely pathogenic variants and other frameshifts, start losses or gains, and ± 1, 2 splice site variants were manually reviewed by a board-certified individual in molecular diagnostics by the American Board of Clinical Chemistry (T.P.S.) and a genetic counselor (K.C.B.) to establish a final set of putative mutations (our summary term for likely pathogenic and pathogenic variants[22]). During manual review, ClinVar (National Center for Biotechnology Information, Bethesda, MD) was used to confirm final determinations when available for a particular variant. For variants that lacked consensus among ClinVar commercial laboratories (Online Mendelian Inheritance in Man excluded), Ingenuity Variant Analysis classification was considered confirmed if consistent with at least one of the following hereditary gene testing laboratories: Ambry Genetics (Aliso Viejo, CA), Invitae (San Francisco, CA), GeneDx (Gaithersburg, MD), or the Sharing Clinical Reports Project (Myriad Genetics, Salt Lake City, UT). Last exon protein-truncating variants were called as likely pathogenic or pathogenic unless located after a known benign polymorphic stop codon in ClinVar. Appendix Table A1 (online only) includes a final list of the putative mutations by individual.

Statistical Analysis

Data are presented as means or proportions. Descriptive statistics were used to analyze clinical, demographic, and genetic test result characteristics; χ^2 tests were used to compare groups. A two-sided $P < .05$ was considered statistically significant.

RESULTS

More than 50 cancer types were studied, including lung (41%), breast (19%), colorectal (8%), prostate (6%), pancreas (3%), and ovarian (2%). Average patient age was 63.5 years (range, 18 to 95 years), and 43% were male. Overall, 239 potential germline mutations in 227 unique patients were identified. Of these, 83 (34.7%) in 74 patients were excluded because of high ctDNA load, which made germline-somatic origin unclear (see Variant Curation in Patients and Methods).

Of the 83 excluded mutations, 44 (53%) were in *TP53*, 20 (24.1%) in *APC*, nine (10.8%) in *RB1*, four (4.8%) in *PTEN*, and two (2.4%) each in *CDKN2A*, *NF1*, and *STK11*. Excluded variants generally were congruent with the known somatic mutation spectrum of their respective tumor types: 77% of excluded *TP53* alterations (n = 34) were in lung, breast, or colorectal cancer (CRC); 75% of excluded *APC* alterations (n = 15) were in CRC; 33% of excluded *RB1* alterations (n = 3) were in non–small-cell lung cancer (which potentially indicates small-cell transformation), and one was in small cell lung cancer; and 50% of excluded *PTEN* mutations (n = 2) were in breast cancer, one in melanoma and one in non–small-cell lung cancer. No mutations in *ATM*, *BRCA1*, *BRCA2*, *RET*, *MLH1*, or *SMAD4* were excluded because of high ctDNA fraction.

In total, 156 mutations in 156 patients (1.4%) were considered likely germline (Table 1; Appendix Tables A1 and A2, online only), with *BRCA1* and *BRCA2* mutations being the most common (78% combined [n = 122]). Eighty-eight (82.2%) of 107 unique mutations were previously identified in ClinVar as likely pathogenic or pathogenic. Only one putative germline mutation implicated in Lynch syndrome was identified most likely because only a single Lynch syndrome gene exon (exon 12 of *MLH1*) was included in Guardant360.

Putative germline mutation identification was consistent among 12 individuals with multiple samples evaluated. Serial sampling MAFs ranged from 42.4% to 56.1%, with the largest intrapatient variation being 7.3% (Appendix Table A2).

Overall, the highest prevalence of putative germline mutations was in patients with ovarian (8.1%), prostate (3.5%), pancreatic (3.3%), and breast (2.2%) cancer (Table 1). Putative mutation rates were higher in patients younger than 50 years of age across all tumor types (3.0% *v* 1.2% [$P < .001$]; breast cancer excluded, 2.1% *v* 1.2% [$P = .017$]) and only in patients with breast cancer (4.7% *v* 1.4%; $P < .001$).

The mean MAF of putative germline findings was 48.7%, and MAFs ranged from the cohort limit of 40% to 95.6% (Fig 1). Examination of the relationship between germline MAF estimates and copy number variation established that deviation from expected germline allelic frequency (50%) often is concordant with copy number estimates (Figs 2 and 3). Putative germline mutations in tumor suppressor genes were observed with ctDNA copy number losses in the same gene (presumably loss of heterozygosity of the wild-type allele). Distinct mechanisms explain secondary hits evident in the data, including gene deletions (ie, loss of heterozygosity) and somatic truncating mutations (Fig 4). In addition, somatic reversion mutations that restore expression of functional proteins were identified, which likely evolved as a resistance mechanism to prior therapy[23] (Fig 4).

Germline testing of leukocyte DNA confirmed germline mutation status in five patients (one with *ATM*, one with *BRCA1*,

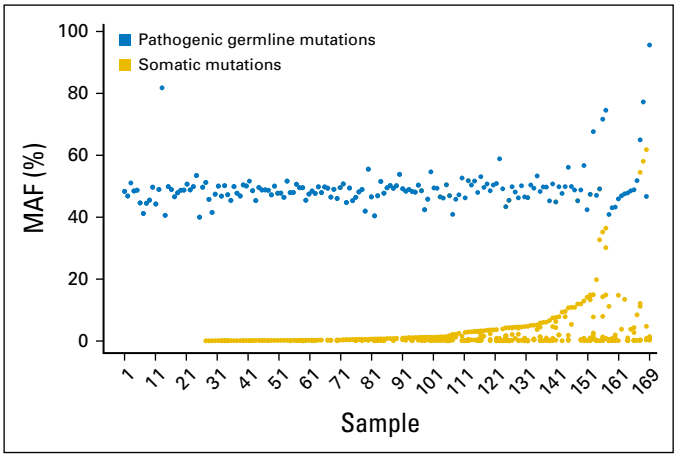


Fig 1. Somatic mutation and putative pathogenic germline mutant allele fractions (MAFs) for all 156 individuals with putative germline mutations arranged by lowest to highest somatic MAF. This included 169 samples in total; 144 individuals had one sample evaluated, and 12 had more than one sample evaluated (25 samples total; Appendix Tables A1 and A2). Putative germline mutations (pathogenic germline) had MAFs close to 50%, whereas somatic mutations had lower MAFs, as expected. Copy number variation corrections account for MAFs greater than 60%. Some samples did not have somatic mutations.

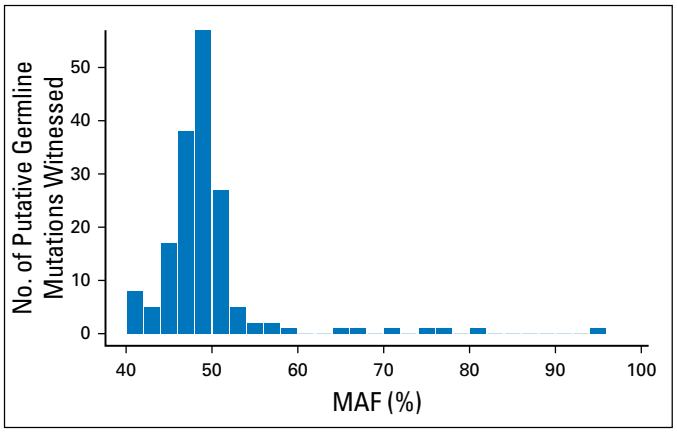


Fig 2. Distribution of mutant allele fractions (MAFs) of putative germline mutations. The majority of mutations had MAFs close to 50%, as expected. Mutations with MAFs less than 40% were not evaluated; those with MAFs greater than 60% were included if concomitant locus copy number loss was identified (see Figs 3 and 4).

three with *BRCA2*) for which ancillary germline testing results were available (100% concordance), and six additional patients (five with *BRCA2*, one with *MLH1*) had congruent germline-positive results by clinician report (no primary germline laboratory report available). In addition, 19 *BRCA* Ashkenazi Jewish founder mutations were identified[24]; most (58% [n = 11 of 19]) were in patients with breast, ovarian, or prostate cancer, consistent with hereditary breast and ovarian cancer syndrome,[3] whereas another six were in patients with lung cancer and one each in patients with neuroendocrine carcinoma and carcinoma of unknown primary. A Brazilian *TP53* p.R337H founder mutation associated with Li-Fraumeni syndrome also was identified in a 38-year-old patient with breast cancer.[25]

DISCUSSION

We leveraged cfDNA NGS analysis to identify and differentiate germline mutations from somatic mutations without a priori knowledge of germline mutation status to identify a spectrum of putative germline findings in a large cohort of patients with advanced solid tumors who underwent somatic genomic testing as part of routine clinical care. This patient cohort primarily represented the four most common cancers in the United States in 2018—breast, lung, prostate, and colorectal—as well as pancreatic cancer, the 11th most common cancer type.[26] Of note, in addition to diseases where genetic counseling and testing recommendations are supported by evidence-based guidelines, we identified putative germline mutations in common tumor types, including lung, pancreatic, and prostate, where routine germline testing is not common, which suggests that incidental putative germline mutation reporting in these cancer types could significantly affect clinical care.

Although we observed a lower prevalence of cancer predisposition germline mutations than previously reported among other advanced cancer cohorts,[7,27-29] we used a conservative approach to establish the final putative germline set, which likely underestimates of the true prevalence of germline mutations. Reasons for the lower prevalence are multiple: Our methods removed mutations with MAFs of less than 40%, although most commercial laboratories would consider genomic DNA mutations of more than 25% MAF to be germline; only a subset of exons were sequenced for seven of the 16 genes; several well-described cancer susceptibility genes were not included in the Guardant360 panel; the cohort was highly enriched for patients with lung cancer, a type not commonly associated with hereditary cancer susceptibility; we did not evaluate germline copy number variation or intragenic rearrangements; and some mutations were excluded because of a high ctDNA fraction.

With regard to mutations excluded because of high ctDNA load, *TP53*, *APC*, *RB1*, and *PTEN* accounted for 93% of the total. These genes frequently are mutated in cancer[27] and often are early somatic events.[30,31] Therefore, somatic mutations in these genes are more likely to have higher MAFs, which leads to an inherent challenge in discerning germline from somatic mutations in the plasma of patients with advanced cancer. In contrast, no putative germline mutations in *ATM*, *BRCA1*, *BRCA2*, *RET*, *MLH1*, or *SMAD4* were excluded as a result of high ctDNA load potentially because these genes are less likely to be early mutations (compared with *TP53*, *APC*, *RB1*, and *PTEN*).[27] Therefore, putative germline findings in *ATM*, *BRCA1*, *BRCA2*, *RET*, *MLH1*, or *SMAD4* from cfDNA analysis may represent more often a true germline mutation, especially if identified in cancers where somatic mutations in these genes are not common. Increasing cfDNA assay coverage of common SNPs in genes associated with hereditary cancer predisposition might improve discrimination of somatic versus germline mutations at high MAFs and improve incidental germline mutation detection rates.

Secondary analyses revealed many important associations and observations. Younger age at diagnosis has been associated with hereditary cancer predisposition germline mutations, and colorectal and breast cancer guidelines frequently recommend

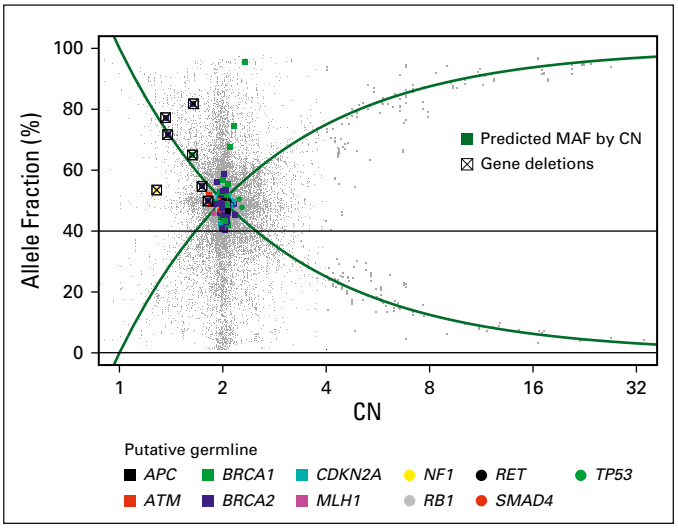
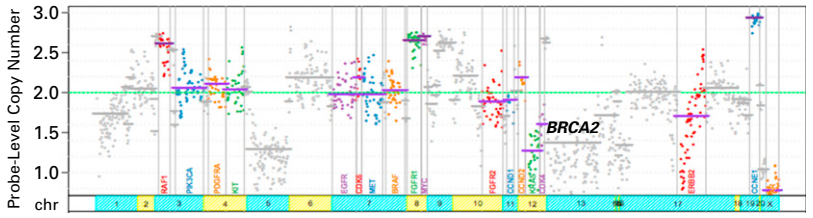


Fig 3. Relationship between mutant allele fractions (MAFs) of identified putative germline mutations and common single nucleotide polymorphisms. The putative germline MAFs are plotted against, and in agreement with, genomic copy number (CN) measured using corresponding single nucleotide polymorphisms at the same allele. Mutations under 40% MAF were not evaluated.

A **Patient With Prostate Cancer: *BRCA2* Germline SNV + Gene Deletion (second hit)**



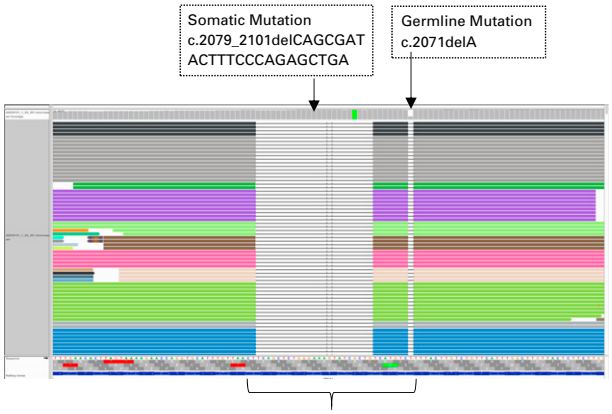
Cancer type	Prostate adenocarcinoma
Germline SNV	c.631+2T>G
Alteration type	Splice variant
Transcript ID	NM_000059.3
Exon	7
Observed MAF	77%
Observed copy number	1.36

B **Patient With Prostate Cancer: *BRCA2* Germline SNV + Somatic SNV (second hit)**

Mutation	Allele Frequency, %	Interpretation
BRCA2 c.2830A>T (p.Lys944*)	49.9	Germline mutation
BRCA2 c.8301delT (p.Glu2769fs)	1.96	Somatic mutation (second hit)

C **Patient With Ovarian Cancer: *BRCA1* Germline SNV + Gene Deletion (second hit) + *BRCA1* Reversion SNV (restores reading frame)**

Mutation	Allele Frequency or Copy Number, %	Interpretation
BRCA1 c.2071delA (p.Arg691fs)	74.5	Germline mutation
BRCA1 gene deletion	1.66	Somatic mutation (second hit)
BRCA1 c.2079_2101del CAGCGATACTTTCCCAGA GCTGA (p.Asp693fs)	2.15	Somatic mutation (reversion mutation)



Taken individually, each mutation results in a frameshift (loss of function). Combined, the net result is a 24-nucleotide deletion that puts *BRCA1* back in frame (ie, reverts to pseudo–wild type).

Fig 4. Three example genetic profiles of patients with secondary tumor mutations. The putative germline mutations identified are presented with any respective *BRCA1* or *BRCA2* circulating tumor findings. (A) Patient with a *BRCA2* gene deletion and concomitant putative germline single nucleotide variant (SNV). (B) Patient with both germline and somatic *BRCA2* SNVs. (C) Patient with a reversion of the germline SNV in the circulating tumor DNA with an allele frequency of 2.15%, potentially explaining the patient's reported resistance to platinum-based chemotherapy (the wild-type allele presumed lost because of a *BRCA1* gene deletion). chr, chromosome; ID, identifier; MAF, mutant allele fractions.

genetic risk assessment and consideration of genetic testing for individuals younger than 50 years of age with a cancer diagnosis.[2,3] In the current study, rates of putative germline mutations were elevated in individuals younger than 50 years, consistent with patients who carry a germline cancer predisposition. In particular, putative germline mutations also were enriched in patients with ovarian cancer, consistent with the relatively high rates of germline mutations found in these patients,[29] which supports recommendations for germline genetic counseling/testing for all individuals with epithelial ovarian cancer.[3]

We also demonstrate the value of gene copy number assessment in the evaluation of putative germline mutations in cfDNA. Because most cancer predisposition genes are autosomal tumor suppressors, two inactivating events generally are needed (one in each allele) to eliminate gene function and initiate tumorigenesis.[32] Therefore, low allele fraction ctDNA abnormalities (mutations or copy number loss) in individuals with a putative germline mutation in the same gene may reveal the likely driver of the cancer.

With regard to therapy, restoration of germline-inactivated alleles (revertant mutations) identifiable with cfDNA NGS, which appear as a second alteration in genomic proximity to a germline or early somatic mutation, can either restore the open reading frame (in cases of frameshift mutations) or alter a nonsense mutation to a missense or synonymous alteration.[33,34] Identification of germline mutations in such contexts is critical for proper therapy selection, particularly when considering poly (ADP-ribose) polymerase inhibitor therapy in the context of *BRCA1* or *BRCA2* mutations.[8,23]

Strengths of our study include large patient numbers and a wide spectrum of advanced cancers. The study was performed at a Clinical Laboratory Improvement Amendments–approved commercial laboratory[35] with rigorous sample handling procedures and analytics. One limitation of the study design is that germline DNA samples (eg, from leukocytes, fibroblasts) for orthogonal validation of germline status were not available. However, all five patients with known ancillary clinical testing of leukocyte DNA had confirmed putative germline mutations, and findings from another six were supported by clinician report. The observed enrichment for known ancestral founder mutations also supports the accuracy of this study because such enrichment would not be expected for mutations of somatic or hematopoietic origin.

Furthermore, most mutations were reported in ClinVar by major hereditary gene testing laboratories, and repeat sampling in 12 patients showed consistency of the putative germline mutation finding.

Another limitation is that the Guardant360 assay does not evaluate for *BRCA*ness/homology–directed repair, microsatellite instability, or tumor mutational burden estimates, which may have been useful in identifying somatic correlations with germline variants. Furthermore, the de-identified methods used did not allow for provider recontact or collection of medical information beyond the test requisition form such that detailed pathology; health habits (eg, smoking history); family history; or race, ancestry, or ethnicity information were not available for study analyses.

The ability of ctDNA testing to identify incidental germline mutations is subject to the same challenges of reporting incidental germline findings as seen in patients who undergo tumor tissue sequencing.[36] Because ctDNA laboratories likely will begin to include incidental germline findings, ordering providers must be knowledgeable about patient family genetic cancer risk assessment and genetic counseling resources (ie, local genetic counseling, telegenetics). Providers and patients also need to know that the majority of alterations in genes associated with cancer susceptibility identified through ctDNA analysis represent somatic events and that only a small fraction of identified alterations are germline. That said, before ordering ctDNA testing, providers will need to inform patients that ctDNA testing may identify incidental germline information. Furthermore, laboratories that provide incidental findings will need to distinguish somatic from probable germline findings clearly and include language about the need for confirmatory testing. They also may want to include information about genetic counseling services and/or education material in testing reports to ensure appropriate patient care. A reasonable paradigm for the return of results already exists in the context of universal screening for Lynch syndrome,[37] wherein post-test referral and genetic counseling are acceptable practices. However, a need for some modifications to this paradigm exists given that the majority of patients who undergo ctDNA testing will have metastatic cancer.

Collectively, our findings and those previously published[12-14] provide evidence that hereditary cancer predisposition germline mutations can be identified accurately from cfDNA. Despite this, incidental cfDNA germline evaluation should not replace validated hereditary cancer gene testing, but it may serve as an important supplement to increase the reach of genetic cancer risk assessment, particularly in populations with specific germline founder mutations or in cancers without clear hereditary predisposition genetic testing guidelines. Ideally, future studies will include prospective germline collection and companion analyses along with enhancements to the bioinformatics pipeline to allow for confident calls of germline findings. Internal validation in future studies will provide a more accurate estimate of the expected germline mutation prevalence by specific tumor type and enhance the understanding of limitations. Examples of improvements needed for accurate ascertainment of germline mutations include methods to identify germline mutations in cfDNA that account for gene copy number variation and high ctDNA load and germline mutations at low allele fractions in plasma. Furthermore, clinical translation of these findings will depend on establishing best practices to counsel patients about the possibility of incidental germline findings and to report potential germline results clearly to clinicians and/or patients in ways that enhance clinical care.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at jco.org.

AUTHOR CONTRIBUTIONS

Conception and design: Thomas P. Slavin, Kimberly C. Banks, Darya Chudova, Geoffrey R. Oxnard, Justin I. Odegaard, Angel A. Rodriguez, Aditya Bardia, Richard B. Lanman, Razelle Kurzrock, Jeffrey N. Weitzel
Financial support: Thomas P. Slavin, Jeffrey N. Weitzel
Administrative support: Brian Leyland-Jones, Christine E. Lee, Jeffrey N. Weitzel
Provision of study materials or patients: Thomas P. Slavin, Massimo Cristofanilli, Angel A. Rodriguez, Mike F. Janicek, Guru Sonpavde, Richard B. Lanman
Collection and assembly of data: Thomas P. Slavin, Kimberly C. Banks, Darya Chudova, Rebecca J. Nagy, Kar Wing Kevin Tsang, Angel A. Rodriguez, Aditya Bardia, Brian Leyland-Jones, Mike F. Janicek, Michael Lilly, Guru Sonpavde, Richard B. Lanman, Jeffrey N. Weitzel
Data analysis and interpretation: Thomas P. Slavin, Kimberly C. Banks, Darya Chudova, Justin I. Odegaard, Susan L. Neuhausen, Stacy W. Gray, Massimo Cristofanilli, Angel A. Rodriguez, Aditya Bardia, Mike F. Janicek, Guru Sonpavde, Christine E. Lee, Richard B. Lanman, Funda Meric-Bernstam, Jeffrey N. Weitzel
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

REFERENCES

1. Lagos-Jaramillo VI, Press MF, Ricker CN, et al: Pathological characteristics of BRCA-associated breast cancers in Hispanics. Breast Cancer Res Treat 130: 281-289, 2011
2. National Comprehensive Cancer Network: NCCN clinical practice guidelines in oncology V.1.2017: Genetic/familial high-risk assessment: Colorectal. https://www.nccn.org/professionals/physician_gls/default.aspx#genetics_colon
3. National Comprehensive Cancer Network: NCCN clinical practice guidelines in oncology V.2.2017: Genetic/familial high-risk assessment: Breast and ovarian. https://www.nccn.org/professionals/physician_gls/default.aspx#genetics_colon
4. Childers CP, Childers KK, Maggard-Gibbons M, et al: National estimates of genetic testing in women with a history of breast or ovarian cancer. J Clin Oncol 35:3800-3806, 2017 [Erratum: J Clin Oncol 36:432, 2018]
5. Mandelker D, Zhang L, Kemel Y, et al: Mutation detection in patients with advanced cancer by universal sequencing of cancer-related genes in tumor and normal DNA vs guideline-based germline testing. JAMA 318:825-835, 2017
6. Huang KL, Mashl RJ, Wu Y, et al: Pathogenic germline variants in 10,389 adult cancers. Cell 173: 355-370, 2018
7. Schrader KA, Cheng DT, Joseph V, et al: Germline variants in targeted tumor sequencing using matched normal DNA. JAMA Oncol 2:104-111, 2016
8. George A, Kaye S, Banerjee S: Delivering widespread BRCA testing and PARP inhibition to

patients with ovarian cancer. Nat Rev Clin Oncol 14: 284-296, 2017

9. Diaz LA Jr, Bardelli A: Liquid biopsies: Genotyping circulating tumor DNA. J Clin Oncol 32: 579-586, 2014

10. Brison N, Van Den Bogaert K, Dehaspe L, et al: Accuracy and clinical value of maternal incidental findings during noninvasive prenatal testing for fetal aneuploidies. Genet Med 19:306-313, 2017

11. Odegaard JI, Vincent JJ, Mortimer S, et al: Validation of a plasma-based comprehensive cancer genotyping assay utilizing orthogonal tissue- and plasma-based methodologies. Clin Cancer Res 24: 3539-3549, 2018

12. Hu Y, Alden RS, Odegaard JI, et al: Discrimination of germline EGFR T790M mutations in plasma cell-free DNA allows study of prevalence across 31,414 cancer patients. Clin Cancer Res 23:7351-7359, 2017

13. Ratajska M, Koczkowska M, Żuk M, et al: Detection of *BRCA1/2* mutations in circulating tumor DNA from patients with ovarian cancer. Oncotarget 8:101325-101332, 2017

14. Shukuya T, Patel S, Shane-Carson K, et al: Lung cancer patients with germline mutations detected by next-generation sequencing and/or liquid biopsy. J Thorac Oncol 13:e17-e19, 2018

15. Lanman RB, Mortimer SA, Zill OA, et al: Analytical and clinical validation of a digital sequencing panel for quantitative, highly accurate evaluation of cell-free circulating tumor DNA. PLoS One 10: e0140712, 2015

16. Zill OA, Mortimer S, Banks KC, et al: Somatic genomic landscape of over 15,000 patients with advanced-stage cancer from clinical next-generation sequencing analysis of circulating tumor DNA. J Clin Oncol 34, 2016 (suppl; abstr LBA11501)

17. Weitzel JN, Chao EC, Nehoray B, et al: Somatic TP53 variants frequently confound germ-line testing results. Genet Med 10.1038/gim.2017.196 [epub ahead of print on November 30, 2017]

18. Hu Y, Ulrich BC, Supplee J, et al: False positive plasma genotyping due to clonal hematopoiesis. Clin Cancer Res 10.1158/1078-0432.CCR-18-0143 [epub ahead of print on March 22, 2018]

19. Pennisi E: Genomics. 1000 Genomes Project gives new map of genetic diversity. Science 330: 574-575, 2010

20. National Heart, Lung, and Blood Institute: NHLBI Grand Opportunity Exome Sequencing Project (ESP), 2016. https://esp.gs.washington.edu/drupal

21. Lek M, Karczewski KJ, Minikel EV, et al: Analysis of protein-coding genetic variation in 60,706 humans. Nature 536:285-291, 2016

22. Richards S, Aziz N, Bale S, et al: Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17:405-424, 2015

23. Benafif S, Hall M: An update on PARP inhibitors for the treatment of cancer. OncoTargets Ther 8:519-528, 2015

24. Hartge P, Struewing JP, Wacholder S, et al: The prevalence of common BRCA1 and BRCA2 mutations among Ashkenazi Jews. Am J Hum Genet 64:963-970, 1999

25. Custódio G, Komechen H, Figueiredo FR, et al: Molecular epidemiology of adrenocortical tumors in southern Brazil. Mol Cell Endocrinol 351:44-51, 2012

26. National Cancer Institute SEER Program: Common Cancer Sites, 2018. https://seer.cancer.gov/statfacts/html/common.html

27. Meric-Bernstam F, Brusco L, Daniels M, et al: Incidental germline variants in 1000 advanced cancers on a prospective somatic genomic profiling protocol. Ann Oncol 27:795-800, 2016

28. Pritchard CC, Mateo J, Walsh MF, et al: Inherited DNA-repair gene mutations in men with metastatic prostate cancer. N Engl J Med 375: 443-453, 2016

29. Walsh T, Casadei S, Lee MK, et al: Mutations in 12 genes for inherited ovarian, fallopian tube, and peritoneal carcinoma identified by massively parallel sequencing. Proc Natl Acad Sci U S A 108:18032-18037, 2011

30. Méniel V, Megges M, Young MA, et al: Apc and p53 interaction in DNA damage and genomic instability in hepatocytes. Oncogene 34:4118-4129, 2015

31. Wolff RK, Hoffman MD, Wolff EC, et al: Mutation analysis of adenomas and carcinomas of the colon: Early and late drivers. Genes Chromosomes Cancer 57:366-376, 2018

32. Knudson AG Jr: Mutation and cancer: Statistical study of retinoblastoma. Proc Natl Acad Sci U S A 68:820-823, 1971

33. Norquist B, Wurz KA, Pennil CC, et al: Secondary somatic mutations restoring BRCA1/2 predict chemotherapy resistance in hereditary ovarian carcinomas. J Clin Oncol 29:3008-3015, 2011

34. Carneiro BA, Collier KA, Nagy RJ, et al: Acquired resistance to poly (ADP-ribose) polymerase inhibitor olaparib in BRCA2-associated prostate cancer resulting from biallelic BRCA2 reversion mutations restores both germline and somatic loss-of-function mutations. JCO Precision Oncol 10.1200/PO.17.00176

35. Schwartz MK: Genetic testing and the clinical laboratory improvement amendments of 1988: Present and future. Clin Chem 45:739-745, 1999

36. Raymond VM, Gray SW, Roychowdhury S, et al: Germline findings in tumor-only sequencing: Points to consider for clinicians and laboratories. J Natl Cancer Inst 108:djv351, 2015

37. Vindigni SM, Kaz AM: Universal screening of colorectal cancers for Lynch syndrome: Challenges and opportunities. Dig Dis Sci 61:969-976, 2016

Affiliations

Thomas P. Slavin, **Kar Wing Kevin Tsang**, **Susan L. Neuhausen**, **Stacy W. Gray**, and **Jeffrey N. Weitzel**, City of Hope, Duarte; **Kimberly C. Banks**, **Darya Chudova**, **Justin I. Odegaard**, **Rebecca J. Nagy**, **Christine E. Lee**, and **Richard B. Lanman**, Guardant Health, Redwood City; **Razelle Kurzrock**, University of California, San Diego, Moores Cancer Center, San Diego, CA; **Geoffrey R. Oxnard**, Dana-Farber Cancer Institute; **Aditya Bardia**, Massachusetts General Hospital Cancer Center and Harvard Medical School, Boston, MA; **Massimo Cristofanilli**, Robert H. Lurie Comprehensive Cancer Center of Northwestern University, Chicago, IL; **Angel A. Rodriguez**, Houston Methodist Hospital; **Funda Meric-Bernstam**, The University of Texas MD Anderson Cancer Center, Houston, TX; **Brian Leyland-Jones**, Avera Cancer Institute, Sioux Falls, SD; **Mike F. Janicek**, Arizona Oncology Associates Gynecology Oncology, Scottsdale, AZ; **Michael Lilly**, Medical University of South Carolina, Charleston, SC; and **Guru Sonpavde**, The University of Alabama at Birmingham Comprehensive Cancer Center, Birmingham, AL.

Support

Supported by National Cancer Institute Grant No. P30CA033572. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Additional support was received from STOP CANCER (T.P.S., principal investigator), the American Cancer Society (J.N.W., principal investigator), and the Avon Foundation (J.N.W., principal investigator).

Prior Presentation

Presented at the 2017 American Society of Clinical Oncology Annual Meeting, Chicago, IL, June 2-6, 2017.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Identification of Incidental Germline Mutations in Patients With Advanced Solid Tumors Who Underwent Cell-Free Circulating Tumor DNA Sequencing

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/jco/site/ifc.

Thomas P. Slavin
No relationship to disclose

Kimberly C. Banks
Employment: Guardant Health
Stock and Other Ownership Interests: Guardant Health
Patents, Royalties, Other Intellectual Property: Guardant Health

Darya Chudova
Employment: Guardant Health
Stock and Other Ownership Interests: Guardant Health
Patents, Royalties, Other Intellectual Property: Guardant Health

Geoffrey R. Oxnard
Honoraria: Chugai Pharma, Bio-Rad, Sysmex, Guardant Health
Consulting or Advisory Role: AstraZeneca, Inivata, Takeda Pharmaceuticals, Loxo Oncology, Ignyta, Dropworks, GRAIL
Patents, Royalties, Other Intellectual Property: Co-author of a patent pending titled "Non-invasive blood-based monitoring of genomic alterations in cancer" (Inst)

Justin I. Odegaard
Employment: Guardant Health
Stock and Other Ownership Interests: Guardant Health

Rebecca J. Nagy
Employment: Guardant Health
Stock and Other Ownership Interests: Guardant Health

Kar Wing Kevin Tsang
No relationship to disclose

Susan L. Neuhausen
No relationship to disclose

Stacy W. Gray
No relationship to disclose

Massimo Cristofanilli
Honoraria: Pfizer
Consulting or Advisory Role: Novartis, Merus

Angel A. Rodriguez
Employment: Austin Cancer Center

Aditya Bardia
Consulting or Advisory Role: Novartis, Genentech, Pfizer, Spectrum Pharmaceuticals, bioTheranostics

Brian Leyland-Jones
Stock and Other Ownership Interests: Catalyst Pharmaceuticals, ProGenix Health Solutions, Puma Biotechnology, Sucampo Pharmaceuticals, ARIAD Pharmaceuticals, Zogenix
Consulting or Advisory Role: GlaxoSmithKline, Amgen
Speakers' Bureau: Genentech, Exelixis, Bayer AG
Research Funding: Takeda Pharmaceuticals, Tesaro
Expert Testimony: Amgen
Travel, Accommodations, Expenses: AKESOgen, National Foundation for Cancer Research

Mike F. Janicek
Stock and Other Ownership Interests: Intuitive Surgical
Honoraria: OncLive
Consulting or Advisory Role: Guardant Health, Ambry Genetics
Speakers' Bureau: Clovis Oncology, AstraZeneca, Tesaro

Michael Lilly
Research Funding: Bavarian Nordic (Inst)

Guru Sonpavde
Honoraria: UpToDate
Consulting or Advisory Role: Bayer AG, Genentech, Sanofi, Merck, Novartis, Agensys, Eisai, AstraZeneca, Janssen Pharmaceuticals, Bristol-Myers Squibb, Exelixis, EMD Serono, Astellas Pharma
Speakers' Bureau: Clinical Care Options, National Comprehensive Cancer Network, Physician Education Resource, OncLive, Research to Practice
Research Funding: Onyx Pharmaceuticals (Inst), Bayer AG (Inst), Boehringer Ingelheim (Inst), Celgene (Inst), Merck (Inst), Pfizer (Inst), Janssen Pharmaceuticals (Inst), Sanofi (Inst)
Other Relationship: Boehringer Ingelheim, AstraZeneca, Bristol-Myers Squibb

Christine E. Lee
Employment: Guardant Health
Stock and Other Ownership Interests: Guardant Health
Patents, Royalties, Other Intellectual Property: Work related to Guardant Health, minor role
Travel, Accommodations, Expenses: Guardant Health

Richard B. Lanman
Employment: Guardant Health, Veracyte
Leadership: Guardant Health, Biolase
Stock and Other Ownership Interests: Guardant Health, Biolase
Research Funding: Guardant Health

Funda Meric-Bernstam
Honoraria: Sumitomo Group, Dialectica
Consulting or Advisory Role: Genentech, Inflection Biosciences, Pieris Pharmaceuticals, Clearlight Diagnostics, DarwinHealth, Samsung Bioepis, Spectrum Pharmaceuticals
Research Funding: Novartis, AstraZeneca, Taiho Pharmaceutical, Genentech, Calithera Biosciences, Debiopharm Group, Bayer AG, Aileron Therapeutics, Puma Biotechnology, CytomX Therapeutics, Jounce Therapeutics, Zymeworks, Curis, Pfizer, eFFECTOR Therapeutics, AbbVie

Razelle Kurzrock
Leadership: CureMatch
Stock and Other Ownership Interests: CureMatch, IDbyDNA
Consulting or Advisory Role: Actuate Therapeutics, Loxo Oncology, XBiotech, NeoMed, Roche
Speakers' Bureau: Roche
Research Funding: Guardant Health (Inst), Sequenom (Inst), Merck Serono (Inst), Genentech (Inst), Pfizer (Inst), Foundation Medicine (Inst), Incyte (Inst), Konica Minolta (Inst)

Jeffrey N. Weitzel
No relationship to disclose

Acknowledgment

We thank Kara Maxwell, MD, PhD, for manuscript planning input.

Appendix

Glossary

American College of Medical Genetics and Genomics variant classification guidelines: standards and guidelines for the interpretation of DNA sequence variants on the basis of a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. The guidelines define five categories for the classification of germline variants (listed from most to least likely to be damaging to the protein): pathogenic, likely pathogenic, variant of uncertain significance, likely benign, and benign. Pathogenic and likely pathogenic variants often are referred to collectively as pathogenic variants or mutations.

Aneuploidy: the presence of an abnormal number of chromosomes in a cell.

Cell-free DNA: all nonencapsulated DNA in the blood stream.

Circulating tumor DNA: tumor-derived cell-free DNA.

Copy number variation: a variation in the number of copies of a DNA segment at a particular genomic position.

Genetic Cancer Risk Assessment: a clinical assessment of a patient's personalized risk for cancer; it commonly includes genetic counseling and germline genetic testing of cancer predisposition genes and provides patient and family recommendations for cancer screening, risk-reducing strategies, and management as appropriate.

Mutant allele fraction: the relative frequency (commonly represented as a percentage) of a mutation at a particular genomic position (locus) determined by next-generation sequencing.

Mutation: a variation in the genetic sequence that usually involves a single nucleotide variation at a specific position in a gene and that is uncommon in the population and believed to disrupt the function of the protein product.

Next-generation sequencing: rapid, high-throughput genome sequencing.

Single nucleotide polymorphism: a variation in a single nucleotide at a specific position in the genome that, in contrast to a mutation, is common in the population.

Single nucleotide variant: a variation in a specific nucleotide at a specific position in the genome, which can represent a single nucleotide polymorphism or mutation.

The Cancer Genome Atlas: a database of DNA and RNA sequencing data and variants from a large collection of tumor samples from patients with a wide range of cancers.

Table A1. Putative Germline Mutations From Individuals With One Sample

Pt ID	Gene	Transcript	Protein Variant	Translational Effect	Allele Frequency, %	Cancer Type	ClinVar ID	dbSNP ID
1	*APC*	NM_000038.5	p.Gln1230Ter	Stop gain	51.06	Endometrial carcinoma	RCV000202019.1	863225344
2	*APC*	NM_000038.5	p.Lys1551Argfs	Stop gain	48.50	Melanoma	RCV000202304.1	NA
3	*APC*	NM_000038.5	p.Lys1551fs	Frameshift	49.92	Gastric adenocarcinoma	NA	NA
4	*APC*	NM_000038.5	p.Ser1415fs	Frameshift	46.71	Colorectal cancer	NA	NA
5	*ATM*	NM_000051.3	c.1027_1030delGAAA	Frameshift	49.83	NSCLC	RCV000236560.2; RCV000122816.7; RCV000129901.4	587780612
6	*ATM*	NM_000051.3	c.1027_1030delGAAA	Frameshift	48.73	Breast cancer	RCV000236560.2; RCV000122816.7; RCV000129901.4	587780612
7	*ATM*	NM_000051.3	c.1027_1030delGAAA	Frameshift	51.83	Pancreatic ductal adenocarcinoma	RCV000236560.2; RCV000122816.7; RCV000129901.4	587780612
8	*ATM*	NM_000051.3	p.Arg3008Cys	Missense	47.44	Lung adenocarcinoma	RCV000169274; RCV000212096; RCV000131173	587782292
9	*ATM*	NM_000051.3	p.Ser3027Lysfs	Frameshift	49.49	Breast cancer	RCV000122895; RCV000220797	NA
10	*BRCA1*	NM_007294.3	p.Arg1835Ter	Stop gain	50.74	Breast cancer	RCV000077627.11; RCV000240766.1; RCV000238956.1; RCV000203652.3; RCV000049020.6; RCV000131862.4	41293465
11	*BRCA1*	NM_007294.3	p.Arg1835Ter	Stop gain	46.57	Breast cancer	RCV000077627.11; RCV000240766.1; RCV000238956.1; RCV000203652.3; RCV000049020.6; RCV000131862.4	41293465
12	*BRCA1*	NM_007294.3	p.Arg1835Ter	Stop gain	47.39	Colorectal cancer	RCV000077627.11; RCV000240766.1; RCV000238956.1; RCV000203652.3; RCV000049020.6; RCV000131862.4	41293465
13	*BRCA1*	NM_007294.3	p.Arg1835Ter	Stop gain	49.70	NSCLC	RCV000077627.11; RCV000240766.1; RCV000238956.1; RCV000203652.3; RCV000049020.6; RCV000131862.4	41293465
14	*BRCA1*	NM_007294.3	p.Arg691Aspfs	Frameshift	74.53	Ovarian carcinoma	RCV000167861.4; RCV000031025.9; RCV000131404.3; RCV000047699.6	80357688
15	*BRCA1*	NM_007294.3	p.Cys61Gly	Missense	40.91	Lung adenocarcinoma	RCV000131902.4; RCV000159935.4; RCV000415051.1; RCV000047597.9; RCV000412714.1; RCV000019229.11; RCV000239114.1	28897672
16	*BRCA1*	NM_007294.3	p.Cys61Gly	Missense	41.95	Melanoma	RCV000131902.4; RCV000159935.4; RCV000415051.1; RCV000047597.9; RCV000412714.1; RCV000019229.11; RCV000239114.1	28897672
17	*BRCA1*	NM_007294.3	p.Gln12Ter	Stop gain	49.64	Pancreatic ductal adenocarcinoma	NA	80357134
18	*BRCA1*	NM_007294.3	p.Gln284Ter	Stop gain	48.54	Ovarian carcinoma	NA	397509330
19	*BRCA1*	NM_007294.3	p.Gln855Ter	Stop gain	44.94	Lung squamous cell carcinoma	RCV000223464.1; RCV000031056.7; RCV000047880.3	80357131
20	*BRCA1*	NM_007294.3	p.Glu1115Terfs	Frameshift	44.61	NSCLC	RCV000048156.2; RCV000077543.6	397509058
21	*BRCA1*	NM_007294.3	p.Glu111Glyfs	Frameshift	53.86	Breast cancer	RCV000195362.2; RCV000031101.7; RCV000239071.1; RCV000048132.5	80357604
22	*BRCA1*	NM_007294.3	p.Glu1257Glyfs	Frameshift	50.43	Ovarian carcinoma	RCV000131814.3; RCV000048325.6; RCV000031127.12; RCV000235232.2	80357993
23	*BRCA1*	NM_007294.3	p.Glu143Ter	Stop gain	46.33	Breast cancer	RCV000162876.3; RCV000031162.8; RCV000074592.7; RCV000465234.1; RCV000048511.4	80356991
24	*BRCA1*	NM_007294.3	p.Glu23Valfs	Frameshift	47.24	Ovarian carcinoma	NA	386833395
25	*BRCA1*	NM_007294.3	p.Glu23Valfs	Frameshift	46.44	Ovarian carcinoma	NA	386833395
26	*BRCA1*	NM_007294.3	p.Glu23Valfs	Frameshift	46.41	Ovarian carcinoma	NA	386833395
27	*BRCA1*	NM_007294.3	p.Glu23Valfs	Frameshift	41.20	Lung adenocarcinoma	NA	386833395
28	*BRCA1*	NM_007294.3	p.Glu23Valfs	Frameshift	56.71	Peritoneal carcinoma	NA	386833395
29	*BRCA1*	NM_007294.3	p.Glu23Valfs	Frameshift	45.40	Neuroendocrine carcinoma	NA	386833395
30	*BRCA1*	NM_007294.3	p.Glu23Valfs	Frameshift	43.24	Breast cancer	NA	386833395
31	*BRCA1*	NM_007294.3	p.Glu23Valfs	Frameshift	45.38	NSCLC	NA	386833395
32	*BRCA1*	NM_007294.3	p.Glu23Valfs	Frameshift	45.52	NSCLC	NA	386833395
33	*BRCA1*	NM_007294.3	p.Glu23Valfs	Frameshift	48.01	Lung adenocarcinoma	NA	386833395
34	*BRCA1*	NM_007294.3	p.Glu23Valfs	Frameshift	44.28	Lung adenocarcinoma	NA	386833395
35	*BRCA1*	NM_007294.3	p.Glu908Ter	Stop gain	50.05	NSCLC	RCV000074576.7; RCV000047943.4; RCV000077527.6; RCV000131878.3; RCV000239255.1; RCV000148387.1	80356978
36	*BRCA1*	NM_007294.3	p.Glu908Ter	Stop gain	51.78	Ovarian carcinoma	RCV000074576.7; RCV000047943.4; RCV000077527.6; RCV000131878.3; RCV000239255.1; RCV000148387.1	80356978
37	*BRCA1*	NM_007294.3	p.Gly1788Val	Missense	44.52	Cholangiocarcinoma	RCV000048961.4; RCV000235698.2; RCV000162885.1; RCV000031241.6	80357069
38	*BRCA1*	NM_007294.3	p.Ile1237Asnfs	Frameshift	51.17	Gastric carcinoma	RCV000130545.2; RCV000048294.2; RCV000112160.4	80357564
39	*BRCA1*	NM_007294.3	p.Leu1679Tyrfs	Frameshift	46.17	Ovarian carcinoma	RCV000031205.6; RCV000048742.3; RCV000483893.1	80357623
40	*BRCA1*	NM_007294.3	p.Leu22Ser	Missense	67.65	Ovarian carcinoma	NA	80357438
41	*BRCA1*	NM_007294.3	p.Lys1037Terfs	Frameshift	48.80	Breast cancer	RCV000031089.6; RCV000130035.2; RCV000048072.2	397507209
42	*BRCA1*	NM_007294.3	p.Lys654Serfs	Frameshift	95.65	Breast cancer	RCV000236783.2; RCV000047660.7; RCV000414241.1; RCV000130764.3; RCV000031019.9	80357522
43	*BRCA1*	NM_007294.3	p.Met1775Arg	Missense	51.69	Breast cancer	RCV000167787.5; RCV000048931.6; RCV000019264.9; RCV000131375.4	41293463
44	*BRCA1*	NM_007294.3	p.Pro1585Argfs	Frameshift	64.99	Ovarian carcinoma	RCV000219878.1; RCV000048652.2; RCV000483332.1; RCV000112394.4	80357837
45	*BRCA1*	NM_007294.3	p.Trp1508Ter	Stop gain	50.38	Breast cancer	RCV000077237.3; RCV000236102.2; RCV000077575.6; RCV000129129.3; RCV000048586.5	80356885
46	*BRCA1*	NM_007294.3	p.Tyr1552Ter	Frameshift	49.34	Ovarian carcinoma	NA	NA
47	*BRCA1*	NM_007294.3	p.Val1736Ala	Missense	49.51	Lung carcinoid	RCV000048857.6; RCV000195366.4; RCV000131291.3; RCV000031229.5	45553935
48	*BRCA2*	NM_000059.3	Splicing c.7806-2A>G	Splicing	51.64	Lung adenocarcinoma	NA	81002836
49	*BRCA2*	NM_000059.3	Splicing c.631+2T>G	Splicing	77.24	Prostate adenocarcinoma	NA	81002899
50	*BRCA2*	NM_000059.3	Splicing c.67+1G>C	Splicing	40.61	Breast cancer	NA	NA
51	*BRCA2*	NM_000059.3	p.Ala1922Cysfs	Frameshift	50.08	Pancreatic ductal adenocarcinoma	RCV000113479.3; RCV000044747.2	NA

(continued on following page)

Table A1. Putative Germline Mutations From Individuals With One Sample (continued)

Pt ID	Gene	Transcript	Protein Variant	Translational Effect	Allele Frequency, %	Cancer Type	ClinVar ID	dbSNP ID
52	*BRCA2*	NM_000059.3	p.Ala938Profs	Frameshift	53.33	Pancreatic ductal adenocarcinoma	RCV000044064.9; RCV000458791.1; RCV000238794.1; RCV000210161.1; RCV000160273.3; RCV000131102.4; RCV000240755.3; RCV000009907.9	80359351
53	*BRCA2*	NM_000059.3	p.Ala938Profs	Frameshift	46.91	Lung adenocarcinoma	RCV000044064.9; RCV000458791.1; RCV000238794.1; RCV000210161.1; RCV000160273.3; RCV000131102.4; RCV000240755.3; RCV000009907.9	80359351
54	*BRCA2*	NM_000059.3	p.Arg2520Ter	Stop gain	48.96	Prostate adenocarcinoma	RCV000045244.6; RCV000217859.2; RCV000162645.3; RCV000210182.1; RCV000077405.6; RCV000148425.1	80358981
55	*BRCA2*	NM_000059.3	p.Arg3052Trp	Missense	40.45	Breast cancer	RCV000210144.1; RCV000045732.4; RCV000163027.2; RCV000221843.2; RCV000077461.5	45580035
56	*BRCA2*	NM_000059.3	p.Arg3128Ter	Stop gain	48.71	Pancreatic ductal adenocarcinoma	RCV000077469.10; RCV000131048.3; RCV000160169.3; RCV000045807.5; RCV000240732.3; RCV000474912.1	80359212
57	*BRCA2*	NM_000059.3	p.Arg3128Ter	Stop gain	47.92	Breast cancer	RCV000077469.10; RCV000131048.3; RCV000160169.3; RCV000045807.5; RCV000240732.3; RCV000474912.1	80359212
58	*BRCA2*	NM_000059.3	p.Asn1626Serfs	Frameshift	46.27	Prostate adenocarcinoma	RCV000131079.3; RCV000160293.3; RCV000031510.9; RCV000044510.6	NA
59	*BRCA2*	NM_000059.3	p.Asn1626Serfs	Frameshift	47.21	Lung adenocarcinoma	RCV000131079.3; RCV000160293.3; RCV000031510.9; RCV000044510.6	NA
60	*BRCA2*	NM_000059.3	p.Asn1784Hisfs	Frameshift	47.47	Melanoma	RCV000044639.5; RCV000031540.9; RCV000131110.3; RCV000074537.8	769126974
61	*BRCA2*	NM_000059.3	p.Asn1933Lysfs	Frameshift	48.17	Breast cancer	RCV000044761.4; RCV000131107.3; RCV000113485.6; RCV000160298.2; RCV000472040.1	NA
62	*BRCA2*	NM_000059.3	p.Asn3124Ile	Missense	48.77	NSCLC	RCV000176516.3; RCV000130337.3; RCV000031816.6; RCV000045802.4	28897759
63	*BRCA2*	NM_000059.3	p.Asp2723His	Missense	49.92	Breast cancer	RCV000131674.3; RCV000074555.7; RCV000077429.6; RCV000045436.6	41293511
64	*BRCA2*	NM_000059.3	p.Asp2723His	Missense	48.40	Endometrial carcinoma	RCV000131674.3; RCV000074555.7; RCV000077429.6; RCV000045436.6	41293511
65	*BRCA2*	NM_000059.3	p.Asp2723His	Missense	49.37	Lung squamous cell carcinoma	RCV000131674.3; RCV000074555.7; RCV000077429.6; RCV000045436.6	41293511
66	*BRCA2*	NM_000059.3	p.Asp2723His	Missense	50.67	Breast cancer	RCV000131674.3; RCV000074555.7; RCV000077429.6; RCV000045436.6	41293511
67	*BRCA2*	NM_000059.3	p.Cys3222Trpfs	Frameshift	46.49	Breast cancer	RCV000130019.3; RCV000114150.3; RCV000045883.2	80359772
68	*BRCA2*	NM_000059.3	p.Gln1429Serfs	Frameshift	53.07	Prostate adenocarcinoma	RCV000044387.6; RCV000031473.11; RCV000130074.4; RCV000160287.3	NA
69	*BRCA2*	NM_000059.3	p.Gln1931Ter	Stop gain	49.13	Peritoneal carcinoma	RCV000077361.5; RCV000131105.3; RCV000044758.2	80358807
70	*BRCA2*	NM_000059.3	p.Gln1987Ter	Stop gain	50.16	Prostate adenocarcinoma	RCV000113510.3; RCV000044803.2; RCV000434288.1	80358828
71	*BRCA2*	NM_000059.3	p.Gln2009Alafs	Frameshift	49.43	Prostate adenocarcinoma	RCV000031596.6; RCV000390850.1; RCV000044820.3; RCV000131918.4	NA
72	*BRCA2*	NM_000059.3	p.Gln397Leufs	Frameshift	48.84	Breast cancer	RCV000238897.2; RCV000043755.5; RCV000131278.2	397515635
73	*BRCA2*	NM_000059.3	p.Gln84Ter	Stop gain	47.95	Breast cancer	RCV000195353.1; RCV000044007.5; RCV000077281.5	80358515
74	*BRCA2*	NM_000059.3	p.Glu1035Ter	Stop gain	43.39	Renal pelvic urothelial carcinoma	RCV000212224.2; RCV000131104.3; RCV000044119.6; RCV000077292.5	80358556
75	*BRCA2*	NM_000059.3	p.Glu2598Ter	Stop gain	46.59	Breast cancer	NA	NA
76	*BRCA2*	NM_000059.3	p.Glu2663Val	Missense	48.11	NSCLC	RCV000163034.1; RCV000077422.5; RCV000257984.2; RCV000484277.1	80359031
77	*BRCA2*	NM_000059.3	p.Glu2846Glyfs	Frameshift	45.47	Prostate adenocarcinoma	RCV000160308.4; RCV000009915.17; RCV000045550.9; RCV000131085.3	80359714
78	*BRCA2*	NM_000059.3	p.Glu2846Glyfs	Frameshift	48.53	Cholangiocarcinoma	RCV000160308.4; RCV000009915.17; RCV000045550.9; RCV000131085.3	80359714
79	*BRCA2*	NM_000059.3	p.Gly2063fs	Frameshift	43.06	Prostate adenocarcinoma	NA	NA
80	*BRCA2*	NM_000059.3	p.Ile1859Lysfs	Frameshift	58.88	Breast cancer	RCV000131118.4; RCV000160296.4; RCV000044684.6; RCV000031556.11	770318608
81	*BRCA2*	NM_000059.3	p.Ile605Asnfs	Frameshift	47.85	NSCLC	RCV000160269.3; RCV000031343.14; RCV000043897.7; RCV000131453.3	768562561
82	*BRCA2*	NM_000059.3	p.Ile605Asnfs	Frameshift	48.30	Prostate adenocarcinoma	RCV000160269.3; RCV000031343.14; RCV000043897.7; RCV000131453.3	768562561
83	*BRCA2*	NM_000059.3	p.Leu1908Argfs	Frameshift	50.65	Prostate adenocarcinoma	RCV000461157.1; RCV000044728.8; RCV000160297.4; RCV000009905.15; RCV000131120.3	80359530
84	*BRCA2*	NM_000059.3	p.Leu2357Valfs	Frameshift	71.65	Breast cancer	RCV000045136.7; RCV000195404.4; RCV000031664.9; RCV000131032.4	756538291
85	*BRCA2*	NM_000059.3	p.Leu2740Ter	Stop gain	48.82	Breast cancer	RCV000045454.2; RCV000009934.6; RCV000113889.2; RCV000236578.1	80359070
86	*BRCA2*	NM_000059.3	p.Lys1530Ter	Stop gain	45.30	Pancreatic ductal adenocarcinoma	RCV000077329.6; RCV000044447.5; RCV000212238.2; RCV000162921.2	80358692
87	*BRCA2*	NM_000059.3	p.Lys2162Asnfs	Frameshift	41.53	Breast cancer	RCV000212249.2; RCV000031630.7; RCV000131034.3; RCV000044967.5	770263702
88	*BRCA2*	NM_000059.3	p.Lys2777Argfs	Frameshift	48.85	NSCLC	RCV000257157.2	NA
89	*BRCA2*	NM_000059.3	p.Lys3296fs*17	Frameshift	49.77	NSCLC	NA	NA
90	*BRCA2*	NM_000059.3	p.Lys585Asnfs	Frameshift	50.17	Ovarian carcinoma	RCV000131055.4; RCV000031335.7; RCV000074514.7; RCV000212215.2	NA
91	*BRCA2*	NM_000059.3	p.Lys944Ter	Stop gain	49.89	Prostate adenocarcinoma	RCV000077287.5; RCV000131101.4; RCV000212222.2; RCV000044070.4	80358533
92	*BRCA2*	NM_000059.3	p.Ser1882Ter	Stop gain	49.53	Breast cancer	RCV000167830.3; RCV000240722.1; RCV000077204.3; RCV000131114.3; RCV000044705.6; RCV000031565.8	80358785

(continued on following page)

Table A1. Putative Germline Mutations From Individuals With One Sample (continued)

Pt ID	Gene	Transcript	Protein Variant	Translational Effect	Allele Frequency, %	Cancer Type	ClinVar ID	dbSNP ID
93	*BRCA2*	NM_000059.3	p.Ser1982Argfs	Frameshift	47.79	Lung adenocarcinoma	RCV000129627.4; RCV000009911.4; RCV000034451.8; RCV000009912.4; RCV000009910.15; RCV000367838.1; RCV000212245.3; RCV000044800.9; RCV000414179.1	80359550
94	*BRCA2*	NM_000059.3	p.Ser1982Argfs	Frameshift	47.75	Ovarian carcinoma	RCV000129627.4; RCV000009911.4; RCV000034451.8; RCV000009912.4; RCV000009910.15; RCV000367838.1; RCV000212245.3; RCV000044800.9; RCV000414179.1	80359550
95	*BRCA2*	NM_000059.3	p.Ser1982Argfs	Frameshift	47.54	Prostate adenocarcinoma	RCV000129627.4; RCV000009911.4; RCV000034451.8; RCV000009912.4; RCV000009910.15; RCV000367838.1; RCV000212245.3; RCV000044800.9; RCV000414179.1	80359550
96	*BRCA2*	NM_000059.3	p.Ser1982Argfs	Frameshift	42.43	Prostate adenocarcinoma	RCV000129627.4; RCV000009911.4; RCV000034451.8; RCV000009912.4; RCV000009910.15; RCV000367838.1; RCV000212245.3; RCV000044800.9; RCV000414179.1	80359550
97	*BRCA2*	NM_000059.3	p.Ser1982Argfs	Frameshift	50.39	Carcinoma of unknown primary	RCV000129627.4; RCV000009911.4; RCV000034451.8; RCV000009912.4; RCV000009910.15; RCV000367838.1; RCV000212245.3; RCV000044800.9; RCV000414179.1	80359550
98	*BRCA2*	NM_000059.3	p.Ser1982Argfs	Frameshift	45.27	Breast cancer	RCV000129627.4; RCV000009911.4; RCV000034451.8; RCV000009912.4; RCV000009910.15; RCV000367838.1; RCV000212245.3; RCV000044800.9; RCV000414179.1	80359550
99	*BRCA2*	NM_000059.3	p.Ser1982Argfs	Frameshift	48.68	Prostate adenocarcinoma	RCV000129627.4; RCV000009911.4; RCV000034451.8; RCV000009912.4; RCV000009910.15; RCV000367838.1; RCV000212245.3; RCV000044800.9; RCV000414179.1	80359550
100	*BRCA2*	NM_000059.3	p.Ser599Terfs	Frameshift	48.93	Pancreatic neuroendocrine tumor	RCV000129987.3; RCV000031337.8; RCV000043887.6; RCV000168232.4	276174813
101	*BRCA2*	NM_000059.3	p.Ser611Ter	Stop gain	50.75	NSCLC	RCV000131056.3; RCV000031345.7; RCV000212216.2; RCV000043906.5; RCV000077210.2	80358474
102	*BRCA2*	NM_000059.3	p.Ser611Ter	Stop gain	81.79	Prostate adenocarcinoma	RCV000131056.3; RCV000031345.7; RCV000212216.2; RCV000043906.5; RCV000077210.2	80358474
103	*BRCA2*	NM_000059.3	p.Ser780Ter	Stop gain	45.43	Pancreatic ductal adenocarcinoma	RCV000241405.2; RCV000129415.3; RCV000484555.1; RCV000238974.1; RCV000077211.2	587781471
104	*BRCA2*	NM_000059.3	p.Thr3033Asnfs	Frameshift	40.89	Breast cancer	RCV000045711.6; RCV000195406.2; RCV000464852.1; RCV000031791.7; RCV000210094.1; RCV000130439.3	754205122
105	*BRCA2*	NM_000059.3	p.Thr441Glnfs	Frameshift	49.17	Lung squamous cell carcinoma	RCV000484432.1	NA
106	*BRCA2*	NM_000059.3	p.Trp1563Ter	Stop gain	48.13	Colorectal cancer	RCV000241224.1	NA
107	*BRCA2*	NM_000059.3	p.Trp1692Metfs	Frameshift	49.00	NSCLC	RCV000130743.4; RCV000031524.9; RCV000160294.3; RCV000238830.1; RCV000044550.8	766647221
108	*BRCA2*	NM_000059.3	p.Trp1692Metfs	Frameshift	50.05	Prostate adenocarcinoma	RCV000130743.4; RCV000031524.9; RCV000160294.3; RCV000238830.1; RCV000044550.8	766647221
109	*BRCA2*	NM_000059.3	p.Trp1692Metfs	Frameshift	49.17	NSCLC	RCV000130743.4; RCV000031524.9; RCV000160294.3; RCV000238830.1; RCV000044550.8	766647221
110	*BRCA2*	NM_000059.3	p.Trp2586Ter	Stop gain	47.89	Thyroid carcinoma	RCV000045303.2; RCV000255811.2; RCV000077214.2; RCV000031698.6	80359004
111	*BRCA2*	NM_000059.3	p.Trp2626Cys	Missense	46.04	NSCLC	RCV000045336.4; RCV000077215.2; RCV000031707.7; RCV000163025.2; RCV000482471.1	80359013
112	*BRCA2*	NM_000059.3	p.Tyr1894Ter	Stop gain	47.75	Breast cancer	RCV000148424.1; RCV000160095.4; RCV000077219.3; RCV000044719.7; RCV000031570.10; RCV000131121.3	41293497
113	*BRCA2*	NM_000059.3	p.Tyr1894Ter	Stop gain	46.85	Breast cancer	RCV000148424.1; RCV000160095.4; RCV000077219.3; RCV000044719.7; RCV000031570.10; RCV000131121.3	41293497
114	*BRCA2*	NM_000059.3	p.Tyr1894Ter	Stop gain	46.94	Breast cancer	RCV000148424.1; RCV000160095.4; RCV000077219.3; RCV000044719.7; RCV000031570.10; RCV000131121.3	41293497
115	*BRCA2*	NM_000059.3	p.Tyr2215Serfs	Frameshift	45.90	Prostate adenocarcinoma	RCV000416517.2; RCV000219562.3; RCV000031642.7; RCV000131027.3	80359616
116	*BRCA2*	NM_000059.3	p.Tyr3098Ter	Stop gain	49.45	Prostate adenocarcinoma	RCV000031812.6; RCV000074562.7; RCV000045784.5; RCV000210096.1; RCV000465472.1; RCV000131041.3	80359200
117	*BRCA2*	NM_000059.3	p.Tyr3098Ter	Stop gain	45.33	Breast cancer	RCV000031812.6; RCV000074562.7; RCV000045784.5; RCV000210096.1; RCV000465472.1; RCV000131041.3	80359200
118	*BRCA2*	NM_000059.3	p.Tyr792Ter	Stop gain	49.56	Breast cancer	RCV000077221.2; RCV000131058.3; RCV000077277.5; RCV000043981.3	80358503
119	*BRCA2*	NM_000059.3	p.Val1283Lysfs	Frameshift	49.37	Prostate adenocarcinoma	RCV000031440.8; RCV000160281.1; RCV000044280.8; RCV000131095.3	746229647
120	*BRCA2*	NM_000059.3	p.Val1283Lysfs	Frameshift	45.85	Pancreatic ductal adenocarcinoma	RCV000031440.8; RCV000160281.1; RCV000044280.8; RCV000131095.3	746229647
121	*BRCA2*	NM_000059.3	p.Val1804Lysfs	Frameshift	54.64	Breast Cancer	RCV000131109.2; RCV000219181.3; RCV000044655.3; RCV000031544.6	NA
122	*BRCA2*	NM_000059.3	p.Val726Serfs	Frameshift	50.35	NSCLC	RCV000031357.6; RCV000240717.1	NA
123	*CDKN2A*	NM_000077.4	p.Arg24Pro	Missense	48.94	Pancreatic ductal adenocarcinoma	RCV000472219.1; RCV000236320.1; RCV000167312.3; RCV000010022.2; RCV000410204.1	104894097
124	*CDKN2A*	NM_000077.4	p.Arg24Pro	Missense	49.81	Cancer, other	RCV000472219.1; RCV000236320.1; RCV000167312.3; RCV000010022.2; RCV000410204.1	104894097
125	*CDKN2A*	NM_000077.4	p.Asp153Tyr	Missense; stop gain	46.84	Adenocarcinoma of unknown primary	RCV000223581.1; RCV000198192.3	45476696
126	*CDKN2A*	NM_000077.4	p.Glu69Gly	Missense; synonymous	51.55	NSCLC	RCV000235616.2; RCV000166237.2; RCV000205699.1	372670098

(continued on following page)

Table A1. Putative Germline Mutations From Individuals With One Sample (continued)

Pt ID	Gene	Transcript	Protein Variant	Translational Effect	Allele Frequency, %	Cancer Type	ClinVar ID	dbSNP ID
127	*CDKN2A*	NM_000077.4	p.Ile49Thr	Missense	48.35	Breast cancer	RCV000412396.1; RCV000122945.5; RCV000212398.2; RCV000115331.6	199907548
128	*CDKN2A*	NM_000077.4	p.Ile49Thr	Missense	50.23	Breast cancer	RCV000412396.1; RCV000122945.5; RCV000212398.2; RCV000115331.6	199907548
129	*CDKN2A*	NM_000077.4	p.Ile49Thr	Missense	50.19	Renal cell carcinoma	RCV000412396.1; RCV000122945.5; RCV000212398.2; RCV000115331.6	199907548
130	*CDKN2A*	NM_000077.4	p.Ile49Thr	Missense	51.24	Cancer, other	RCV000412396.1; RCV000122945.5; RCV000212398.2; RCV000115331.6	199907548
131	*MLH1*	NM_000249.3	p.Lys461Ter	Stop gain	45.80	Colorectal cancer	RCV000202201.3; RCV000030213.3; RCV000132422.3	63750540
132	*NF1*	NM_000267.3	p.Arg440Ter	Stop gain	48.57	Sarcoma	RCV000213237.1; RCV000225855.2	778405030
133	*NF1*	NM_000267.3	p.Phe150fs*15	Frameshift	49.88	Lung adenocarcinoma	NA	NA
134	*NF1*	NM_000267.3	p.Tyr2264Terfs	Frameshift	46.81	Endometrial carcinoma	RCV000486063.1; RCV000213933.1	NA
135	*NF1*	NM_000267.3	p.Tyr489Cys	Missense	53.47	Prostate adenocarcinoma	RCV000000382.6	137854557
136	*RB1*	NM_000321.2	p.Ala15fs*3	Frameshift	40.00	Carcinoma of unknown primary	NA	NA
137	*RB1*	NM_000321.2	p.Gly617fs*36	Frameshift	47.04	Carcinoma of unknown primary	NA	NA
138	*RET*	NM_020975.4	p.Cys609Tyr	Missense	49.84	Pancreatic ductal adenocarcinoma	RCV000424503.1; RCV000431942.1; RCV000444552.1; RCV000441078.1; RCV000168107.3; RCV000014958.26; RCV000173889.3; RCV000021778.1; RCV000082049.6	77939446
139	*RET*	NM_020975.4	p.Cys634Tyr	Missense	47.66	Thyroid carcinoma	RCV000021823.1; RCV000014925.25; RCV000425364.1; RCV000438527.1; RCV000422622.1; RCV000014924.22; RCV000129490.3; RCV000421191.1; RCV000432822.1; RCV000476408.1; RCV000182582.2	75996173
140	*SMAD4*	NM_005359.5	p.Ala451Leufs	Frameshift	46.93	Colorectal cancer	RCV000235213.1; RCV000115881.4	587782540
141	*TP53*	NM_000546.5	p.Arg213Ter	Stop gain	47.76	Breast cancer	NA	397516436
142	*TP53*	NM_000546.5	p.Arg282Gln	Missense	49.39	Carcinoma of unknown primary	RCV000439593.1; RCV000418376.1; RCV000442318.1; RCV000427734.1; RCV000442471.1; RCV000422340.1; RCV000421276.1; RCV000426667.1; RCV000428909.1; RCV000436164.1; RCV000431918.1; RCV000433180.1; RCV000437335.1; RCV000235474.2; RCV000434324.1; RCV000438489.1; RCV000425549.1; RCV000226273.2; RCV000429554.1; RCV000423658.1; RCV000444806.1	NA
143	*TP53*	NM_000546.5	p.Arg337His	Missense	49.71	Breast cancer	RCV000128923.3; RCV000013178.23; RCV000413754.1; RCV000197240.4; RCV000481814.1	121912664
144	*TP53*	NM_000546.5	p.Arg342Ter	Stop gain	50.48	Cancer, other	RCV000213069.3; RCV000161074.4	730882029

NOTE. Pt ID is arbitrary.
Abbreviations: ID, identifier; NA, not applicable; NSCLC, non–small-cell lung cancer; Pt, patient.

Table A2. Putative Germline Mutations From Individuals With More Than One Sample

Pt ID	Gene	Transcript	Protein Variant	Translational Effect	Allele Frequency, %	Cancer Type	ClinVar ID	dbSNP ID
145a	*BRCA1*	NM_007294.3	p.Cys226Valfs	Frameshift	52.68	Breast cancer	RCV000235776.1; RCV000049091.4; RCV000031274.9; RCV000129754.3	80357941
145b	*BRCA1*	NM_007294.3	p.Cys226Valfs	Frameshift	55.51	Breast cancer	RCV000235776.1; RCV000049091.4; RCV000031274.9; RCV000129754.3	80357941
146a	*BRCA1*	NM_007294.3	p.Met1775Arg	Missense	46.58	Ovarian carcinoma	RCV000167787.5; RCV000048931.6; RCV000019264.9; RCV000131375.4	80357061
146b	*BRCA1*	NM_007294.3	p.Met1775Arg	Missense	50.89	Ovarian carcinoma	RCV000167787.5; RCV000048931.6; RCV000019264.9; RCV000131375.4	80357061
147a	*BRCA1*	NP_009225.1	p.Ser1383Ter	Stop gain	48.79	Ovarian carcinoma	RCV000077233.2; RCV000213696.1; RCV000483963.1; RCV000112270.3; RCV000048458.2	80357071
147b	*BRCA1*	NP_009225.1	p.Ser1383Ter	Stop gain	50.76	Ovarian carcinoma	RCV000077233.2; RCV000213696.1; RCV000483963.1; RCV000112270.3; RCV000048458.2	80357071
148a	*BRCA2*	NM_000059.3	p.Asn3124Ile	Missense	48.98	Prostate adenocarcinoma	RCV000176516.3; RCV000130337.3; RCV000031816.6; RCV000045802.4	28897759
148b	*BRCA2*	NM_000059.3	p.Asn3124Ile	Missense	45.75	Prostate adenocarcinoma	RCV000176516.3; RCV000130337.3; RCV000031816.6; RCV000045802.4	28897759
149a	*BRCA2*	NM_000059.3	p.Ser1982Argfs	Frameshift	56.12	Breast cancer	RCV000129627.4; RCV000009911.4; RCV000034451.8; RCV000009912.4; RCV000009910.15; RCV000367838.1; RCV000212245.3; RCV000044800.9; RCV000414179.1	80359550
149b	*BRCA2*	NM_000059.3	p.Ser1982Argfs	Frameshift	48.78	Breast cancer	RCV000129627.4; RCV000009911.4; RCV000034451.8; RCV000009912.4; RCV000009910.15; RCV000367838.1; RCV000212245.3; RCV000044800.9; RCV000414179.1	80359550
150a	*BRCA2*	NM_000059.3	p.Glu2198Asnfs	Frameshift	49.86	Breast cancer	RCV000044989.6; RCV000131037.3; RCV000215210.1; RCV000009904.12	80359605
150b	*BRCA2*	NM_000059.3	p.Glu2198Asnfs	Frameshift	44.77	Breast cancer	RCV000044989.6; RCV000131037.3; RCV000215210.1; RCV000009904.12	80359605
151a	*BRCA2*	NM_000059.3	p.Ala938Profs	Frameshift	48.51	Breast cancer	RCV000044064.9; RCV000458791.1; RCV000238794.1; RCV000210161.1; RCV000160273.3; RCV000131102.4; RCV000240755.3; RCV000009907.9	80359351
151b	*BRCA2*	NM_000059.3	p.Ala938Profs	Frameshift	47.98	Breast cancer	RCV000044064.9; RCV000458791.1; RCV000238794.1; RCV000210161.1; RCV000160273.3; RCV000131102.4; RCV000240755.3; RCV000009907.9	80359351
152a	*BRCA2*	NM_000059.3	p.Tyr2215Serfs	Frameshift	46.27	Breast cancer	RCV000416517.2; RCV000219562.3; RCV000031642.7; RCV000131027.3	80359616
152b	*BRCA2*	NM_000059.3	p.Tyr2215Serfs	Frameshift	49.78	Breast cancer	RCV000416517.2; RCV000219562.3; RCV000031642.7; RCV000131027.3	80359616
153a	*BRCA2*	NM_000059.3	p.Gln1235Ter	Stop gain	47.27	NSCLC	NA	NA
153b	*BRCA2*	NM_000059.3	p.Gln1235Ter	Stop gain	48.44	NSCLC	NA	NA
154a	*CDKN2A*	NM_000077.4	p.Ile49Thr	Missense	47.68	NSCLC	RCV000412396.1; RCV000122945.5; RCV000212398.2; RCV000115331.6	199907548
154b	*CDKN2A*	NM_000077.4	p.Ile49Thr	Missense	42.41	NSCLC	RCV000412396.1; RCV000122945.5; RCV000212398.2; RCV000115331.6	199907548
155a	*CDKN2A*	NM_000077.4	p.Ile49Thr	Missense	48.35	Breast cancer	RCV000412396.1; RCV000122945.5; RCV000212398.2; RCV000115331.6	199907548
155b	*CDKN2A*	NM_000077.4	p.Ile49Thr	Missense	49.73	Breast cancer	RCV000412396.1; RCV000122945.5; RCV000212398.2; RCV000115331.6	199907548
155c	*CDKN2A*	NM_000077.4	p.Ile49Thr	Missense	49.60	Breast cancer	RCV000412396.1; RCV000122945.5; RCV000212398.2; RCV000115331.6	199907548
156a	*TP53*	NM_000546.5	p.Asp186fs	Frameshift	50.42	Breast cancer	NA	NA
156b	*TP53*	NM_000546.5	p.Asp186fs	Frameshift	46.56	Breast cancer	NA	NA

NOTE. Pt ID is arbitrary, and numbering continues from Appendix Table A1. The notations a, b, and c refer to serial samples from the same patient at different time points.

Abbreviations: ID, identifier; NA, not applicable; NSCLC, non–small cell lung cancer; Pt, patient.