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Pan-Cancer Analysis of *BRCA1* and *BRCA2* Genomic Alterations and Their Association With Genomic Instability as Measured by Genome-Wide Loss of Heterozygosity

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abstract

PURPOSE BRCA1 or BRCA2 loss of function results in homologous recombination deficiency (HRD), which is targetable by poly (ADP-ribose) polymerase (PARP) inhibitors and other DNA-damaging agents. In cancers associated with germline *BRCA1/2* alterations (*BRCA1/2*-associated cancers: breast, ovarian, pancreatic, prostate), *BRCA1/2* alterations result in HRD and are biomarkers for PARP inhibitor use. In other (non–*BRCA1/2*-associated) cancer types, the association between *BRCA1/2* alteration and HRD is less clear.

METHODS A total of 234,154 tumor samples were sequenced by hybrid capture-based comprehensive genomic profiling. Somatic, germline, and zygosity status was determined computationally. *BRCA1/2* alterations were classified as predicted germline/somatic and biallelic/monoallelic. Genome-wide loss of heterozygosity (gLOH) was evaluated as a marker of HRD.

RESULTS *BRCA1/2* alterations were observed at a 4.7% frequency. *BRCA1/2* mutations were predicted germline in 57.4% of *BRCA1/2*-associated and 37.2% of non–*BRCA1/2*-associated cancers. The fraction of *BRCA1/2*-altered cases that were biallelic was 68.7%, with a higher biallelic fraction in *BRCA1/2*-associated (89.9%) versus non–*BRCA1/2*-associated cancers (43.6%). Differences in tissue distribution of biallelic *BRCA1* versus *BRCA2* alterations were noted, including a higher rate of biallelic *BRCA2* alteration in prostate cancer. Biallelic *BRCA1/2* alteration was observed at a 3.2% frequency (*BRCA1/2*-associated cancers, 8.9%; non–*BRCA1/2*-associated cancers, 1.3%) and > 1% frequency in at least 13 cancer types. Across cancer types, biallelic *BRCA1/2* alteration was associated with increased gLOH versus monoallelic or wild-type *BRCA1/2*; predicted germline or somatic mutations were both associated with elevated gLOH.

CONCLUSION Biallelic *BRCA1/2* alterations were associated with elevated gLOH in diverse cancer types, including those not traditionally associated with *BRCA1/2* cancer syndromes. Biomarker development for PARP inhibitors should integrate methods to distinguish biallelic from monoallelic *BRCA1/2* status, and biallelic *BRCA1/2* alteration should be broadly evaluated across cancer types as a biomarker for underlying HRD and PARP inhibitor sensitivity.

JCO Precis Oncol 4:442-465. © 2020 by American Society of Clinical Oncology

ASSOCIATED CONTENT

Appendix

Data Supplement

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

Accepted on February 10, 2020 and published at ascopubs.org/journal/po on April 30, 2020: DOI https://doi.org/10.1200/PO.19.00345

INTRODUCTION

BRCA1 and *BRCA2* encode critical components of the homologous recombination (HR) DNA repair pathway that maintains genomic stability.[1] Germline *BRCA1/2* (g*BRCA1/2*) alterations are associated with elevated risk for breast, ovarian, pancreatic, and prostate cancer (*BRCA1/2*-associated cancers),[2,3] and tumors that arise in *BRCA1/2* mutation carriers have often lost the wild-type allele.[4] Synthetic lethal interactions between *BRCA1/2* loss of function and poly (ADP-ribose) polymerase inhibitors (PARPi) underlie development and regulatory approval of PARPi targeted therapy for ovarian, breast, and pancreatic cancer.[1,5] Companion diagnostic testing for g*BRCA1/2* and somatic *BRCA1/2* (s*BRCA1/2*) alteration[1] can guide PARPi therapy selection.

BRCA1 or *BRCA2* loss-of-function results in HR deficiency (HRD) and accumulation of chromosomal rearrangements and copy number alterations. *BRCA1* and *BRCA2* have distinct functions in the homology-mediated repair process, and their inactivation leads to different patterns of rearrangements, with *BRCA1* loss of function associated with tandem duplications and *BRCA2* loss of function associated with deletions.[6,7]

CONTEXT

Key Objective

BRCA1/2 loss-of-function alterations result in homologous recombination deficiency (HRD) and are biomarkers for poly (ADP-ribose) polymerase (PARP) inhibitor sensitivity in breast, ovarian, prostate, and pancreatic cancer. To determine the relevance of *BRCA1/2* alterations across cancer types, we evaluated the pan-cancer landscape of *BRCA1/2* alterations and their association with the genome-wide loss-of-heterozygosity (gLOH) marker of HRD.

Knowledge Generated

The fraction of *BRCA1/2* alterations that were biallelic differed by cancer type and predicted germline/somatic status. *BRCA1/2* alterations were most frequently biallelic in breast, ovarian, prostate, and pancreatic cancer; in other cancer types, 44% of *BRCA1/2* alterations were biallelic. Across cancer types, biallelic *BRCA1/2* alteration was associated with elevated gLOH compared with monoallelic or wild-type *BRCA1/2*; this association with HRD was observed irrespective of predicted germline or somatic status.

Relevance

BRCA1/2 biallelic alteration is associated with HRD across tumor types and should be broadly evaluated as a biomarker in trials of PARP inhibitors and other agents that target HRD.

However, the genomic impact of either *BRCA1* or *BRCA2* loss of function can be measured using the genome-wide loss-of-heterozygosity (gLOH) signature of HRD.[1,8] In clinical trials of ovarian cancer, high gLOH (gLOH-high) was associated with improved benefit from the PARPi rucaparib; therefore, gLOH measurement may guide therapeutic decision making.[9,10]

Emerging data from clinical trials suggest that *BRCA1/2* genomic alteration status may also be a predictive biomarker for PARPi in prostate cancer.[11-13] However, PARPi has limited activity in other cancer types with *BRCA1/2* alteration.[14-16] Here, we assessed a genomic data set of 234,154 tumor specimens to determine the landscape of *BRCA1/2* biallelic alterations and their association with gLOH to understand the potential clinical relevance of *BRCA1/2* alterations across cancer types.

METHODS

Approval for this study, including a waiver of informed consent and Health Insurance Portability and Accountability Act waiver of authorization, was obtained from the Western Institutional Review Board (protocol #20152817). Comprehensive genomic profiling (CGP) using hybrid capture-based next-generation sequencing (NGS) was performed on tumor tissue specimens (N = 234,154) submitted to Foundation Medicine during routine clinical care[17] (December 2013-March 2019; Data Supplement). *BRCA1/2* genomic alterations were defined (Appendix) as likely pathogenic alterations or variants of unknown significance (VUSs; not counted as *BRCA1/2* alterations). Zygosity and somatic/germline status for mutations was computationally predicted without matched normal tissue; in validation testing of 480 tumor-only predictions against matched normal specimens, accuracy was 95% for somatic and 99% for germline predictions.[18] *BRCA1/2* alterations were categorized as biallelic (mutations with LOH of the wild-type allele,[18] homozygous deletion, or two or more *BRCA1* or *BRCA2* alterations in a sample), monoallelic (heterozygous mutations with retained wild-type allele),[18] or unknown (alterations where zygosity status could not be determined). Percent gLOH was calculated as a signature of HRD as previously described.[9,10] See the Appendix for full methods.

RESULTS

To assess the prevalence of *BRCA1/2* genomic alterations across cancer types, we examined CGP results from 234,154 tumors sequenced as part of routine clinical care. Overall, *BRCA1/2* alterations were observed in 4.7% of cases (*BRCA1*, 2.1%; *BRCA2*, 2.7%; Fig 1A; Appendix Fig A1). As expected, *BRCA1/2* alterations were most frequently identified in *BRCA1/2*-associated cancers (*BRCA1/2*-associated group, 9.9%; ovarian, 15.2%; prostate, 10.7%; breast, 8.8%; pancreatic, 5.2%). *BRCA1* and *BRCA2* alterations were most frequent in ovarian (10.5%) and prostate cancer (9.6%), respectively; unlike *BRCA2*, *BRCA1* alterations were infrequent in prostate cancer. *BRCA1/2* homozygous deletions were infrequent except in prostate cancer, where *BRCA2* deletions were observed at a 2.6% frequency and accounted for 25% of *BRCA1/2*-altered cases. Across non–*BRCA1/2*-associated cancers, *BRCA1/2* alterations were observed at a 3.0% frequency overall and > 1% frequency in each individual cancer type assessed. *BRCA1/2* mutations were distributed throughout the length of each gene, and most were truncating events (Appendix Fig A2).

Germline/somatic status for *BRCA1/2* short variant mutations was predicted using validated computational methods.[18] We also evaluated performance of germline/somatic predictions in this study. First, in a subset of 23 tumor samples from Rutgers Cancer Institute of New Jersey that arose in patients with g*BRCA1/2* variants identified by genetic testing,

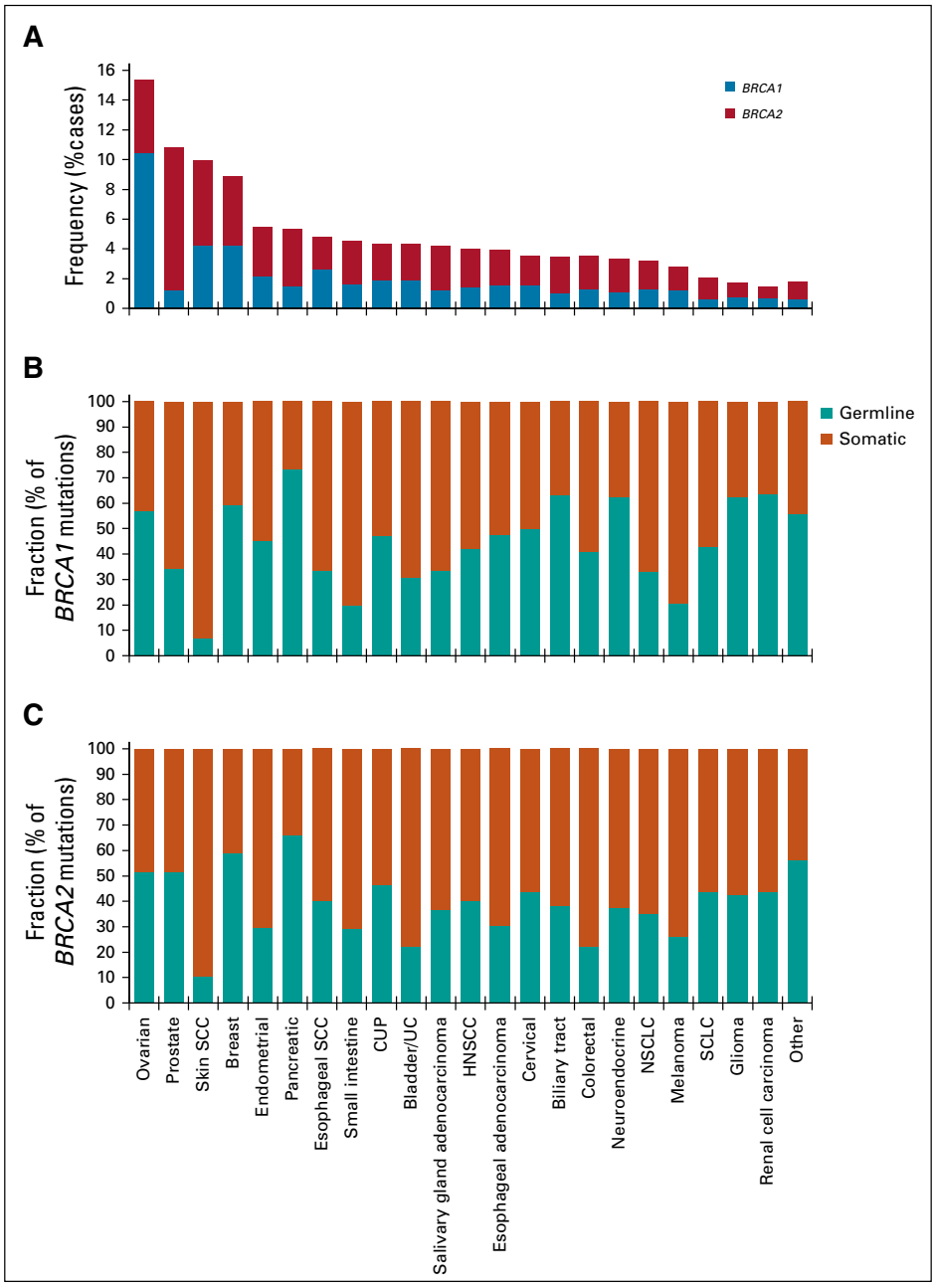

FIG 1. Pan-cancer landscape of *BRCA1/2* alterations. (A) Frequency of *BRCA1* and *BRCA2* alterations across multiple cancer types. Cancer types with ≥ 40 *BRCA1/2*-altered cases are shown, including ovarian cancer (n = 14,256), prostate cancer (n = 7,185), skin squamous cell carcinoma (SCC; n = 661), breast cancer (n = 21,164), endometrial cancer (n = 7,182), pancreatic cancer (n = 12,248), esophageal SCC (n = 836), small intestine cancer (n = 1,145), cancer of unknown primary (CUP; n = 11,130), bladder/urothelial cancer (UC; n = 4,718), salivary gland adenocarcinoma (n = 1,075), head and neck SCC (HNSCC; n = 2,921), gastric/esophageal adenocarcinoma (n = 8,061), cervical cancer (n = 1,694), biliary tract cancer (n = 6,003), colorectal cancer (n = 25,784), neuroendocrine cancer (n = 4,573), non–small-cell lung cancer (NSCLC; n = 43,242), melanoma (n = 6,016), small-cell lung cancer (SCLC; n = 2,262), glioma (n = 8,635), and renal cell carcinoma (n = 3,330); all other cancer types were analyzed as a group labeled other (n = 40,033). See the Data Supplement for detailed data. (B and C) Predicted germline/somatic status was determined computationally for *BRCA1/2* short variant mutations. Fraction (%) of (B) *BRCA1* or (C) *BRCA2* mutations predicted to be germline *v* somatic was determined for each cancer type. See the Data Supplement for detailed data.

computational methods correctly identified 21 (91%) as predicted germline variants. Second, because cell-free DNA (cfDNA) sequencing can often distinguish germline from somatic variants,[19] we evaluated 52 *BRCA1/2* germline/somatic predictions from tissue samples and evaluated patient-matched cfDNA NGS results. Overall, 98.1% of mutations (51 of 52) were observed in cfDNA at allele frequencies consistent with germline/somatic predictions from tumor-only sequencing (Appendix Figs A3A and A3B).

Overall, 47.8% of *BRCA1/2* mutations (*BRCA1*, 51.6%; *BRCA2*, 45.3%) were predicted to be germline (Figs 1B and 1C; Appendix Figs A3C-A3E). As expected, the majority of mutations were predicted to be germline in *BRCA1/2*-associated cancers (*BRCA1*, 58.1%; *BRCA2*, 56.8%), but predicted s*BRCA1/2* mutations comprised an appreciable proportion of *BRCA1/2* mutations. In prostate cancer, 51.7% of *BRCA2 v* 34.4% of *BRCA1* mutations were predicted to be germline. In non–*BRCA1/2*-associated cancer types, *BRCA1/2* mutations were less frequently predicted to be germline (37.2%). Predicted somatic mutations were frequent in skin squamous cell carcinoma (SCC) and melanoma, which accounted for 90.7% and 75.8% of *BRCA1/2* mutations, respectively. As expected, predicted s*BRCA1/2* mutations in these cancer types were often found in a mutational context of ultraviolet light exposure (skin SCC, 45% [14 of 31]; melanoma, 81% [50 of 62]).

We determined whether *BRCA1/2* alterations were likely to affect a single allele (monoallelic) or both alleles (biallelic;

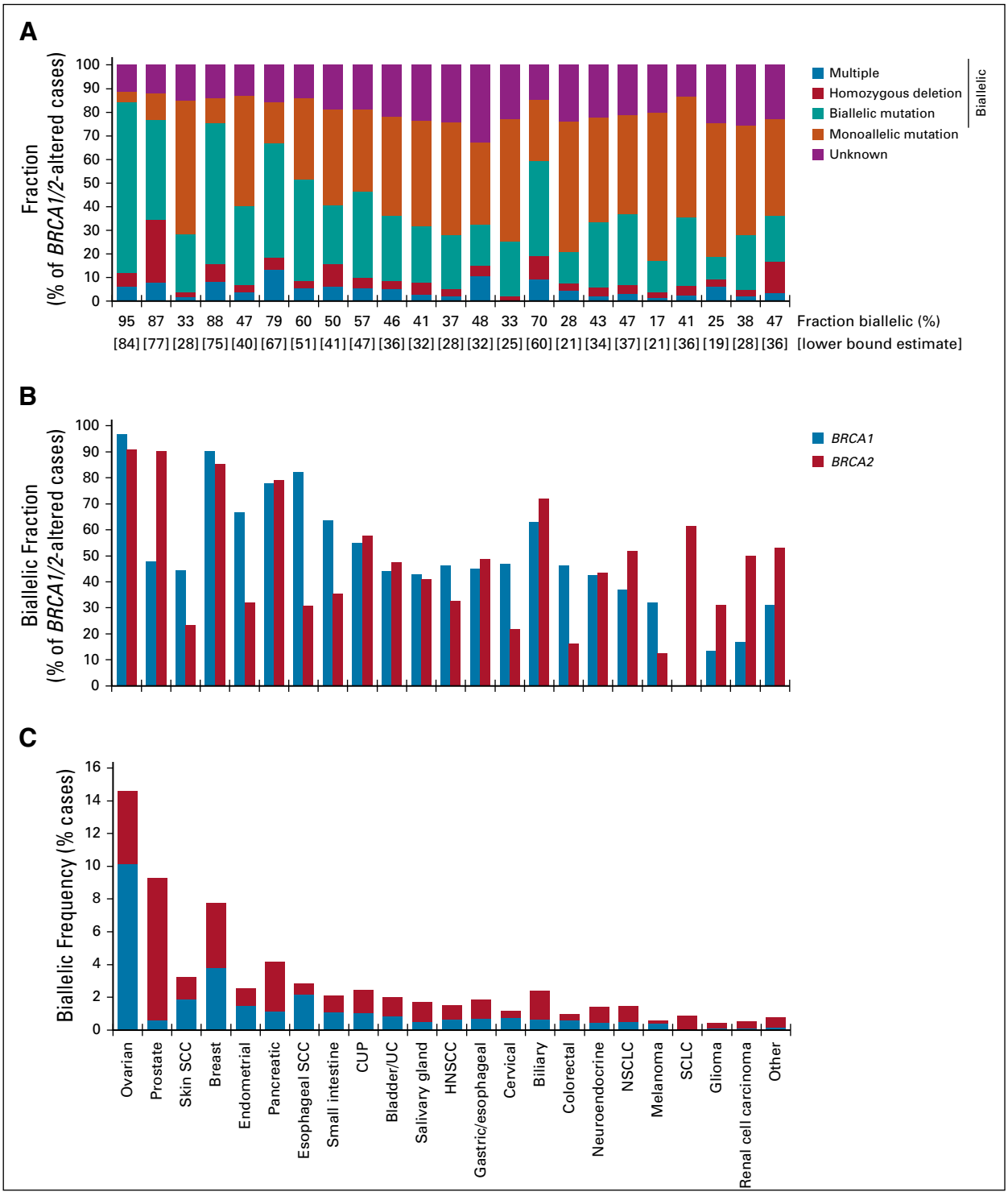

FIG 2. Assessment of *BRCA1* and *BRCA2* biallelic status. (A) Fraction of *BRCA1/2*-altered cases with biallelic or monoallelic alteration was determined. *BRCA1/2*-altered cases were evaluated for class of alteration identified and classified as biallelic (multiple *BRCA1* or multiple *BRCA2* alterations in the same sample, homozygous deletion, biallelic short variant mutation [loss of heterozygosity of the wild-type allele]), monoallelic (heterozygous mutation), or unknown (zygosity status could not be determined). Biallelic fraction (percentage of *BRCA1/2*-altered cases with biallelic alteration) was determined for cases where biallelic/monoallelic status could be called. A lower-bound estimate was established by assessing biallelic cases as a fraction of all *BRCA1/2*-altered cases, including those with unknown biallelic/monoallelic status (see Data Supplement). (B) Biallelic fraction was compared for *BRCA1- v BRCA2*-altered cases for each cancer type (see Data Supplement). (C) Overall frequency of biallelic *BRCA1* and *BRCA2* alterations across multiple cancer types (see Data Supplement). CUP, cancer of unknown primary; HNSCC, head and neck squamous cell carcinoma; NSCLC, non–small-cell lung cancer; SCC, squamous cell carcinoma; SCLC, small-cell lung cancer; UC, urothelial cancer.

Fig 2A; Appendix Fig A4). For cases where biallelic/monoallelic status could be determined, we estimated the fraction of *BRCA1/2*-altered cases with biallelic alteration (biallelic fraction). *BRCA1/2* biallelic fraction was 68.7% overall and highest in *BRCA1/2*-associated cancers (*BRCA1/2*-associated group, 89.9%; ovarian, 94.9%; prostate, 87.3%; breast, 87.9%; pancreatic, 79.4%). Although biallelic fraction was lower in non–*BRCA1/2*-associated cancers ($P <$ .0001, Fisher's exact test), biallelic alterations nonetheless comprised 43.6% of *BRCA1/2*-altered cases: Biallelic fraction was > 50% for esophageal SCC, cancer of unknown primary, and biliary tract cancer.

Biallelic fraction was compared for *BRCA1* versus *BRCA2* (Fig 2B). *BRCA2* biallelic fraction was greater than *BRCA1* in prostate cancer (odds ratio [OR], 10.1; fold difference, 1.9; $P = 1.5 \times 10^{-11}$) and small-cell lung cancer (biallelic fraction, 61.5% *v* 0% for *BRCA2* *v* *BRCA1*, respectively; $P = 2.8 \times 10^{-4}$), whereas *BRCA1* biallelic fraction was greater than *BRCA2* in endometrial cancer (OR, 4.2; fold difference, 2.1; $P = 8.4 \times 10^{-8}$), esophageal SCC (OR, 10.5; fold difference, 2.7; $P = .008$), colorectal cancer (OR, 4.5; fold difference, 2.9; $P = 7.1 \times 10^{-13}$), and melanoma (OR, 3.3; fold difference, 2.6; $P = .02$).

To determine the frequency of *BRCA1/2* loss of function across cancer types, we evaluated biallelic *BRCA1/2*-altered cases as a percentage of all cases (Fig 2C; Appendix Figs A4C-A4F). Biallelic *BRCA1/2* alteration was found in 3.2% of all cases and greatest in *BRCA1/2*-associated cancers (8.9%). Although occurring at a lower frequency in non–*BRCA1/2*-associated cancers (1.3%), biallelic *BRCA1/2* alteration was observed at > 1% frequency in at least 13 cancer types.

Predicted g*BRCA1/2* and s*BRCA1/2* mutations were separately assessed for biallelic fraction (Fig 3; Appendix Fig A5). Overall, 75.4% of predicted g*BRCA1/2* mutations were biallelic *v* 48.5% of s*BRCA1/2* mutations. For *BRCA1/2*-associated cancers, both predicted g*BRCA1/2* (90.8%) and s*BRCA1/2* (81.2%) mutations were frequently biallelic, whereas in non–*BRCA1/2*-associated cancers, fewer predicted g*BRCA1/2* (46.4%) and s*BRCA1/2* (25.4%) mutations were biallelic. Among *BRCA1/2*-associated cancers, cancer type–specific differences were observed. In ovarian and breast cancer, the majority of *BRCA1* and *BRCA2* mutations were biallelic, both for predicted germline and somatic mutations. In prostate cancer, the majority of *BRCA2* (g*BRCA2*, 87.6%; s*BRCA2*, 75.0%) mutations were biallelic, but *BRCA1* (g*BRCA1*, 40.0%; s*BRCA1*, 22.2%) mutations were less frequently biallelic. In pancreatic cancer, predicted g*BRCA1/2* mutations were frequently biallelic (g*BRCA1*, 79.2%; g*BRCA2*, 79.7%) compared with s*BRCA1/2* mutations (s*BRCA1*, 52.9%; s*BRCA2*, 46.0%). Among non–*BRCA1/2*-associated cancers, predicted g*BRCA1* mutations were most frequently biallelic in endometrial (87.1%), unknown primary (66.7%), bladder/urothelial (63.6%), and neuroendocrine (60.0%) cancer; predicted g*BRCA2* mutations were most frequently biallelic in salivary gland (85.7%), unknown primary (71.7%), biliary tract (65.0%), and endometrial (58.1%) cancer; and predicted s*BRCA1/2* mutations were infrequently biallelic except for biliary tract cancer (s*BRCA1*,

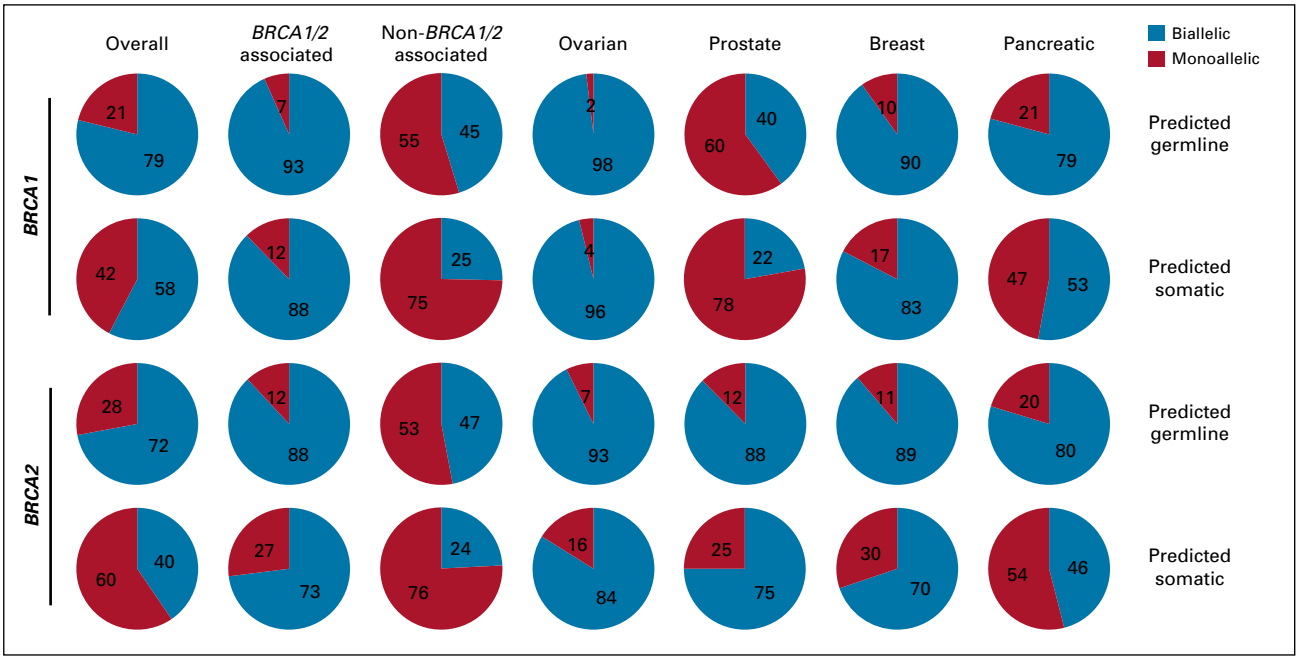

FIG 3. Biallelic and monoallelic distribution for germline *BRCA1/2* and somatic *BRCA1/2* mutations. *BRCA1* and *BRCA2* mutations with a germline or somatic prediction were evaluated for biallelic/monoallelic status for all cancers, *BRCA1/2*-associated cancers (as a group and as individual cancer types), and non–*BRCA1/2*-associated cancers (see Data Supplement).

50.0% [2 of 4]; s*BRCA2*, 61.5% [24 of 39]), endometrial cancer (s*BRCA1*, 48.7% [19 of 39]), and esophageal SCC (s*BRCA1*, 80% [4 of 5]; Appendix Figs A5B and A5C).

We next determined whether *BRCA1/2* status broadly associated with the gLOH signature of HRD. Cases with ≥ 16% gLOH were classified as gLOH-high on the basis of the cutoff established in the ARIEL3 trial of rucaparib in ovarian cancer.[10] Biallelic *BRCA1/2* alterations were associated with gLOH-high across every cancer type examined. The fraction of cases that were gLOH-high was significantly increased for biallelic *BRCA1/2*-altered compared with wild-type cases (Fig 4A, Appendix Fig A6A), whereas the fraction of monoallelic *BRCA1/2* cases that were gLOH-high was similar to wild type. Significant association of biallelic but not monoallelic alterations with gLOH-high was observed both for *BRCA1* and for *BRCA2* when assessed individually (Figs 4B and 4C). Biallelic *BRCA1/2* mutations were associated with gLOH-high irrespective of predicted germline or somatic status (Appendix Figs A6B and A6C). In some cancer types, significant but modest elevation in tumor mutational burden (TMB) was observed for biallelic *BRCA1/2*-mutated cases (predicted germline or somatic) versus wild type; however, the association with TMB was not consistent across cancer types (Appendix Fig A7). Monoallelic s*BRCA1/2*-mutated (but not g*BRCA1/2*) cases were commonly associated with elevated TMB versus wild type, and such mutations may be a consequence of increased mutation rate.

Although biallelic *BRCA1/2* status was consistently associated with gLOH-high, the magnitude was variable for each cancer type, with the greatest association observed in pancreatic (OR, 22.5), biliary (OR, 21.5), endometrial (OR, 17.2), unknown primary (OR, 16.1), and ovarian (OR, 14.9) cancer (Fig 4A). More than 75% of cases with biallelic *BRCA1/2* alterations were gLOH-high for ovarian, breast, pancreatic, unknown primary, and endometrial cancer, whereas fewer than half were gLOH-high for prostate and colorectal cancer. Conversely, > 25% of *BRCA1/2* wild-type cases were gLOH-high for ovarian, breast, lung, and gastric/esophageal cancer.

The ≥ 16% gLOH-high cutoff was clinically validated in ovarian cancer[9,10] and requires optimization for other cancer types. Therefore, gLOH was also assessed as a continuous variable (Figs 5A-5C). Consistent with the findings using a gLOH cutoff-based approach, gLOH scores were higher in *BRCA1/2* biallelic versus wild-type cases across all cancer types evaluated; increased gLOH score was also observed when biallelic *BRCA1* and *BRCA2* were evaluated independently.

HRD signatures (including gLOH-high) may identify additional *BRCA1/2* wild-type tumors potentially suitable for PARPi.[9,10,20] Overall, 19.3% of cases were gLOH-high compared with 3.2% of cases with biallelic *BRCA1/2* alteration, and for most cancer types, the frequency of gLOH-high was greater than biallelic *BRCA1/2* alterations (Data Supplement); distribution of gLOH scores varied between cancer types. To inform rational cancer type–specific gLOH-high cutoffs, we assessed the performance of different gLOH-high thresholds to classify biallelic *BRCA1/2* compared with wild-type cases. Plotting sensitivity to detect cases with biallelic *BRCA1/2* alteration and specificity (percentage of *BRCA1/2* wild-type cases negative for the gLOH-high biomarker), we identified a cutoff that maximized the combined sensitivity and specificity score (Fig 6). For most cancers, the gLOH-high cutoff ranged between 14% and 16%, except for prostate (8.8% cutoff), breast (16.6% cutoff), biliary tract (17.6% cutoff), and gastric (16.7% cutoff) cancer. The identified cutoff for ovarian cancer was 15.1%, which was consistent with the 14% and 16% cutoffs identified in clinical trials.[9,10]

DISCUSSION

The development of PARPi for *BRCA1/2* altered ovarian, breast, and pancreatic cancer[1,5] is predicated on synthetic lethality interactions between *BRCA1/2* loss of function and PARPi/trapping. Emerging clinical trial data suggest that *BRCA1/2* alteration may also be predictive of PARPi response in prostate cancer.[1,11-13] Although responses to PARPi have been documented in other cancer types,[14,21-23] the relevance of *BRCA1/2* alterations in non–*BRCA1/2*-associated cancers remains unclear.[4]

To understand the landscape and phenotypic consequence of *BRCA1/2* alterations, we assessed our data set of 234,154 cancer specimens sequenced using a clinical-grade CGP assay. *BRCA1/2* alterations were frequent in *BRCA1/2*-associated cancers but also observed in a significant fraction (3%) of non–*BRCA1/2*-associated cancers. Predicted germline mutations comprised the majority of *BRCA1/2* mutations in *BRCA1/2*-associated cancers; however, it is notable that 43% were predicted somatic (of which 81% were biallelic) given data that support s*BRCA1/2* alteration as a biomarker for PARPi in ovarian and prostate cancer.[1,11-13]

BRCA1/2 alterations were assessed for biallelic versus monoallelic status to distinguish likely loss of function from biologically neutral alterations. Of note, although monoallelic alteration in *BRCA1/2* mutation carriers may have a subtle haploinsufficient phenotype,[24] it does not lead to severe HRD or sensitivity to platinum-based chemotherapy.[25] Consistent with the established role of *BRCA1/2* in the pathogenesis of *BRCA1/2*-associated cancers, 90% of *BRCA1/2*-altered cases were biallelic with high biallelic fraction both for predicted g*BRCA1/2* and s*BRCA1/2*. In non–*BRCA1/2*-associated cancers, biallelic inactivation still occurred in a significant portion (44%) of *BRCA1/2*-altered cases. Differences in *BRCA1* and *BRCA2* biallelic fraction were observed: *BRCA2* was more frequently biallelic in prostate and small-cell lung cancer, and *BRCA1* was more frequently biallelic in endometrial cancer, esophageal SCC, colorectal cancer, and melanoma. These

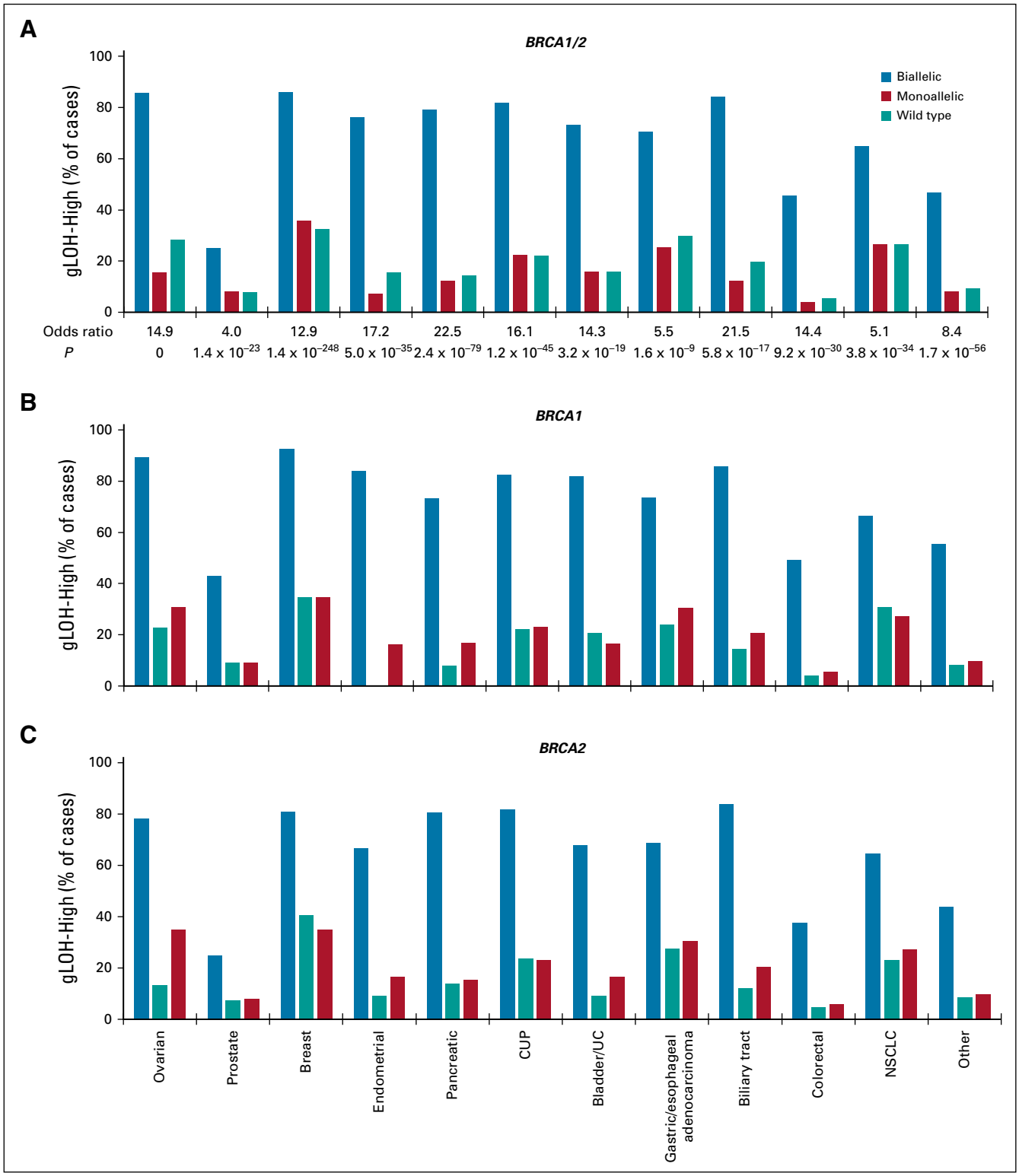

FIG 4. Association between *BRCA1/2* alteration status and high genome-wide loss of heterozygosity (gLOH-high). The frequency of (A) *BRCA1/2*, (B) *BRCA1*, or (C) *BRCA2* biallelic, monoallelic, and wild-type cases that were gLOH-high was compared across cancer types. Cancer types with > 50 biallelic *BRCA1/2*-altered samples were assessed individually, and all other cancer types were grouped together and analyzed as a single group (other). Odds ratios and *P* values (Fisher's exact test) were for comparisons between biallelic and wild-type *BRCA1/2* cases (see Data Supplement). gLOH-high, was defined as ≥ 16% genome-wide loss of heterozygosity cutoff. CUP, cancers of unknown primary; NSCLC, non–small-cell lung cancer; UC, urothelial cancer.

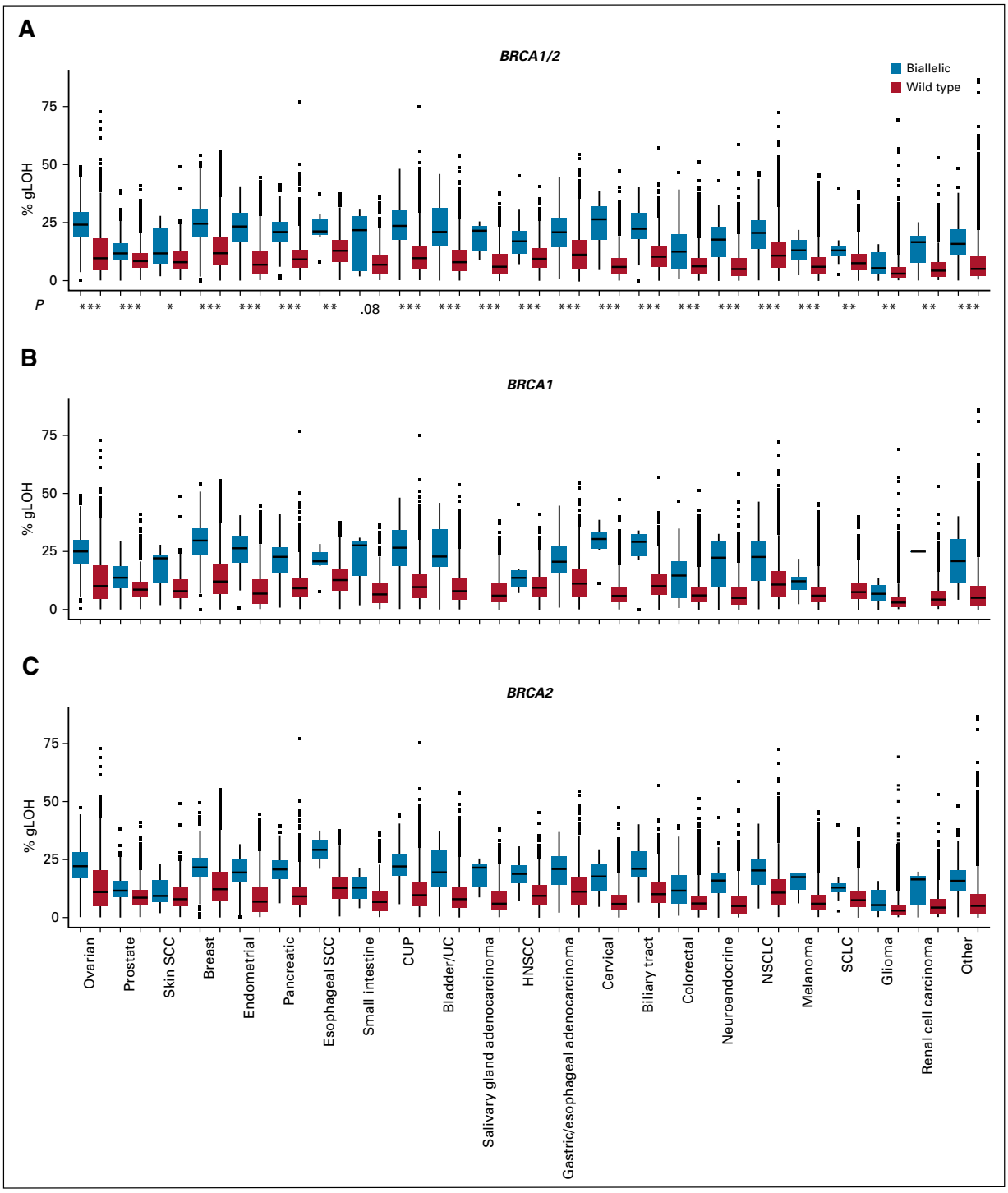

FIG 5. Association between *BRCA1/2* biallelic alteration and genome-wide loss of heterozygosity (gLOH) score as a continuous variable. gLOH score was assessed in (A) *BRCA1/2*, (B) *BRCA1*, and (C) *BRCA2* biallelic *v* wild-type cases across cancer types. Boxes span the first and third quartiles, and the median is denoted by the horizontal line in the box; whiskers indicate maximum and minimum values within 1.5× the interquartile range; black dots indicate outlier events. *P* values are by Mann-Whitney *U* test for comparison between biallelic and wild-type *BRCA1/2* cases. $*P < .05$, $**P < .01$, $***P < .001$ (see Data Supplement). CUP, cancer of unknown primary; HNSCC, head and neck squamous cell carcinoma; NSCLC, non–small-cell lung cancer; SCC, squamous cell carcinoma; SCLC, small-cell lung cancer; UC, urothelial cancer.

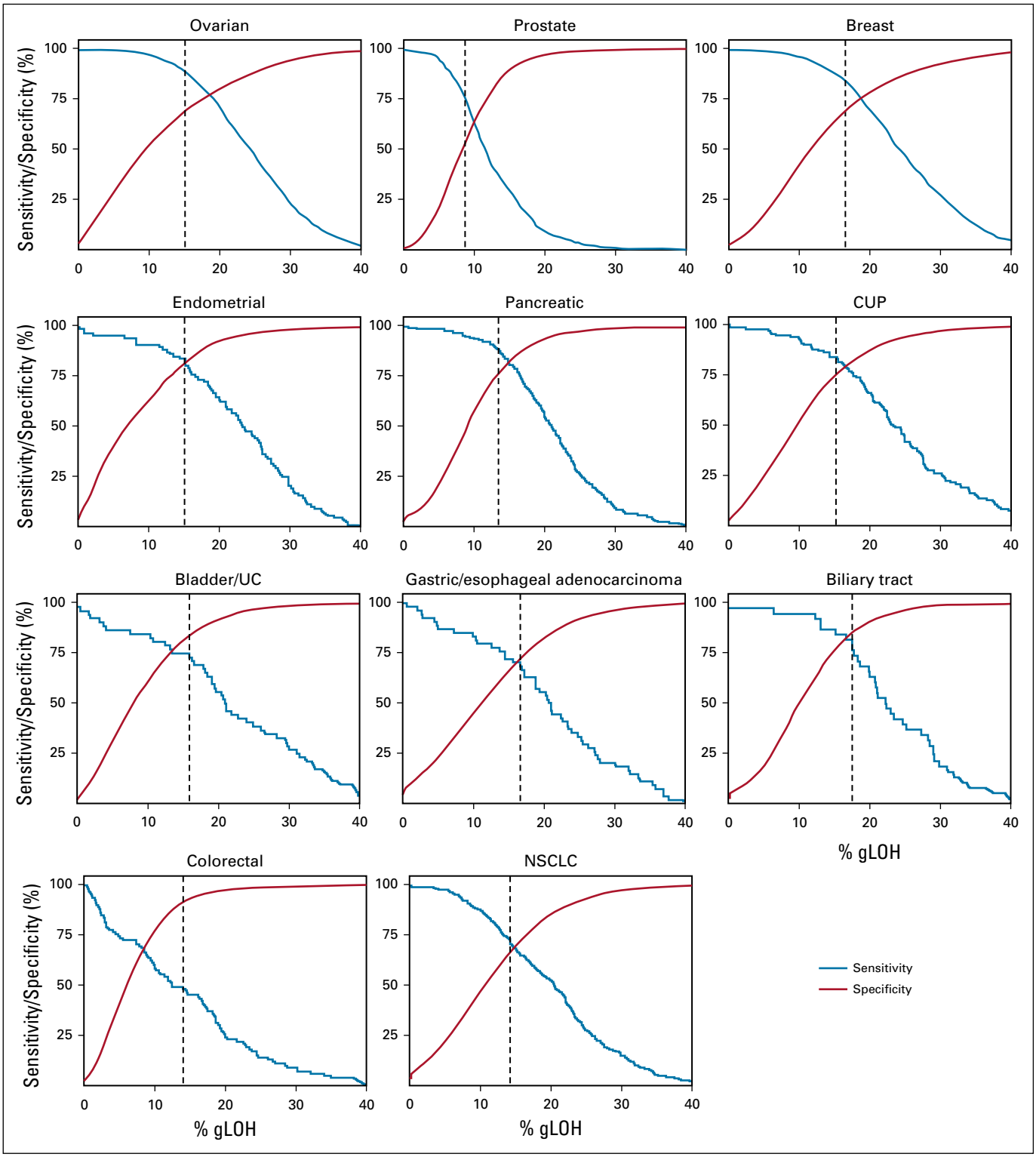

FIG 6. Sensitivity and specificity to classify *BRCA1/2* biallelic alterations *v* wild type were evaluated for different genome-wide loss of heterozygosity (gLOH) cutoffs. Sensitivity was defined as percentage of biallelic *BRCA1/2* cases that were of high gLOH (gLOH-high). Specificity was defined as percentage of *BRCA1/2* wild-type cases that were negative for the gLOH-high biomarker. A cutoff that maximized the combined sensitivity and specificity score was identified (dotted black line; see Data Supplement). CUP, cancer of unknown primary; NSCLC, non–small-cell lung cancer.

differences suggest that *BRCA1* and *BRCA2* may have different tissue specificities outside breast and ovarian cancer.

To determine whether *BRCA1/2* alterations lead to HRD in both *BRCA1/2*-associated and non–*BRCA1/2*-associated cancers, we evaluated gLOH in *BRCA1/2*-altered versus wild-type cases. Across every cancer type evaluated, biallelic *BRCA1/2* alteration was associated with increased gLOH. Therefore, biallelic *BRCA1/2* alterations broadly result in the gLOH signature for HRD and may represent a therapeutic vulnerability targetable by PARPi. In contrast, monoallelic *BRCA1/2* alterations were not associated with elevated gLOH and are likely biologically neutral for HR. Monoallelic alterations may be found in sporadic cancers from g*BRCA1/2* carriers or as somatic passenger mutations.

Distinguishing biallelic from monoallelic status may be an important consideration for refining *BRCA1/2* alteration as a predictive biomarker for PARPi. In *BRCA1/2*-associated cancers, *BRCA1/2* alteration status alone has proven sufficient as a predictive biomarker,[1] likely explained by the majority of *BRCA1/2* alterations in this context being biallelic. Nevertheless, PARPi trials in prostate cancer have incorporated *BRCA1/2* biallelic status into biomarker development,[13] and our finding of significantly lower biallelic fraction for *BRCA1* (*v BRCA2*) in prostate cancer suggests that integrating biallelic status could refine predictive biomarkers in this setting.

The lack of PARPi clinical activity in non–*BRCA1/2*-associated cancers reported previously could be explained by grouped analysis of biallelic and monoallelic *BRCA1/2* alterations.[4] Because of lower rates of biallelic alteration in non–*BRCA1/2*-associated cancers, PARPi clinical trials will likely require patient selection strategies that incorporate methods to discriminate biallelic from monoallelic *BRCA1/2* alterations.

Our findings are consistent with a recent study that demonstrated an elevated HRD composite score in biallelic but not heterozygous *BRCA1/2* cases relative to wild type in an aggregate set of non–*BRCA1/2*-associated cancers.[4] A strength of the current study was that the large data set size enabled analysis of biallelic *BRCA1/2* separately from monoallelic alterations, independent assessment of *BRCA1* and *BRCA2*, and evaluation of non–*BRCA1/2*-associated cancers as individual cancer types rather than in aggregate, which may have enabled identification of associations between biallelic *BRCA1/2* alterations and an HRD signature across cancer types not previously described.[4]

If *BRCA1/2* biallelic alterations functionally result in HRD, they should represent a targetable vulnerability to PARPi and platinum-based chemotherapy, irrespective of whether they are drivers of disease pathogenesis or bystander passenger alterations.[26] Our data demonstrate that biallelic *BRCA1/2* alteration in non–*BRCA1/2*-associated cancers are associated with the gLOH-high signature of HRD and, therefore, warrant investigation in PARPi trials. Biallelic *BRCA1/2* alteration was observed in 1.6% of non–*BRCA1/2*-associated cancer and at > 1% frequency in at least 13 cancer types. Although biallelic *BRCA1/2* alterations occur at low prevalence in non–*BRCA1/2*-associated cancers, basket trials that led to the tumor-agnostic approvals of NTRK inhibitors for *NTRK* fusion-positive tumors and pembrolizumab for microsatellite instability–high tumors[27] demonstrate feasibility of therapeutic development for rare pan-cancer biomarkers. Clinical trials such as the TAPUR study (ClinicalTrials.gov identifier: NCT02693535) that includes a PARPi treatment arm for *BRCA1/2*-altered cancers will inform PARPi development in non–*BRCA1/2*-associated cancers, and analyses may benefit from consideration of monoallelic versus biallelic status.

Although gLOH-high is associated with clinical benefit from rucaparib in ovarian cancer,[9,10] understanding of the gLOH biomarker in other cancer types is required. Evaluation of sensitivity and specificity of varying gLOH thresholds to distinguish biallelic *BRCA1/2*-altered and wild-type cases may inform development of disease-specific gLOH-high cutoffs. In future studies, analysis of *BRCA1/2* wild-type, gLOH-high cases may be a discovery tool for characterizing *BRCA1/2* VUSs and for prioritizing candidate non-*BRCA1/2* HR pathway gene biomarkers.[9] Although detection of gLOH-high in *BRCA1/2* wild-type cases potentially expands the patient population addressable by PARPi, the utility of gLOH-high requires validation in prospective trials for nonovarian cancers.

Limitations of this study should be acknowledged. First, we focus on gLOH as a biomarker for HRD; other HRD markers were not evaluated, including telomeric allelic imbalance (TAI), large-scale transition (LST), myChoice HRD (combination LOH/TAI/LST score; Myriad Genetics, Salt Lake City, UT), Signature 3, and HRDetect.[7,8,28-33] In PARPi trials, HRD biomarkers have focused on approaches that are compatible with targeted NGS assays (gLOH, myChoice HRD), which are used in routine clinical practice.[1,9,10] In contrast, Signature 3 and HRDetect signatures have been evaluated in the research setting using whole-exome or whole-genome sequencing; novel methods may enable future clinical assessment of Signature 3 with targeted assays.[34] Second, germline/somatic status predictions using tumor-only sequencing and computational methods are less definitive compared with matched normal sequencing used in other studies.[4,18] Third, using *BRCA1/2* biallelic versus wild-type status to refine gLOH-high cutoffs is confounded by some *BRCA1/2* wild-type gLOH-high cases that are HRD because of *BRCA1/2* alteration–independent mechanisms, such as *BRCA1/2* methylation or alteration in other HR genes. Another study evaluated a group of 102 HR pathway genes and found that biallelic alterations were associated with HRD.[35] Other HR pathway genes sequenced here could inform gLOH-high thresholds

in the future; however, we focused on *BRCA1/2* because other HR pathway genes have not been consistently predictive of clinical response to PARPi,[11-13,36] and robust clinical evidence supporting predictive biomarker genes beyond *BRCA1/2* is lacking. Finally, clinical outcomes data that associate PARPi response with biallelic *BRCA1/2* alteration and gLOH were not available and require evaluation in clinical trials.

We demonstrate that biallelic *BRCA1/2* alterations are associated with elevated gLOH across all cancer types evaluated and may therefore represent a therapeutic vulnerability targetable by PARPi. Biomarker development for PARPi should reliably distinguish biallelic/monoallelic *BRCA1/2* status, and biallelic *BRCA1/2* alteration should be broadly evaluated as a pan-cancer biomarker in PARPi clinical trials.

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PRIOR PRESENTATION

Presented at the European Society of Medical Oncology 2018 Congress, Munich, Germany, October 19-23, 2018.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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No other potential conflicts of interest were reported.

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APPENDIX

Supplementary Methods

Approval for this study, including a waiver of informed consent and Health Insurance Portability and Accountability Act waiver of authorization, was obtained from the Western Institutional Review Board (IRB; protocol #20152817). Comprehensive genomic profiling (CGP) was performed using hybrid capture-based next-generation sequencing (NGS) (median coverage, > 790×; Data Supplement) in a Clinical Laboratory Improvement Amendments–certified, College of American Pathologists–accredited, New York State–approved laboratory (Foundation Medicine; Frampton GM, et al: Nat. Biotechnol 31: 1023-1031, 2013). CGP results included in this study were from tumor tissue specimens (N = 234,154) submitted as part of routine clinical care (December 2013-March 2019); for patient characteristics, see the Data Supplement. Results were analyzed for base substitutions, short insertions/deletions, rearrangements, and copy number alterations. *BRCA1/2* genomic alterations were defined as likely pathogenic alterations (protein-truncating mutations/rearrangements [except for *BRCA2* truncations that occur at K3326 or downstream], homozygous deletions, or characterized missense mutations [mutations designated as pathogenic in BRCA Exchange/ENIGMA consortium (Cline MS, et al: PLoS Genet doi:10.1371/journal.pgen.1007752, 2018) or consensus pathogenic in ClinVar, mutations included in the ARIEL3 trial (Coleman RL, et al: Lancet 390:1949-1961, 2017), and functionally characterized mutations]); other alterations that were classified as variants of unknown significance (VUSs) were not included as *BRCA1/2* genomic alterations in the analysis. Zygosity and somatic/germline status for mutations were computationally predicted without matched normal tissue. In validation testing of 480 tumor-only sequencing calls against matched normal specimens, accuracy was 95% for somatic and 99% for germline calls (Sun JX, et al: PLOS Comput Biol 14: e1005965, 2018); in assessment of zygosity calls, significant enrichment in mutations with loss of heterozygosity (LOH) was observed for tumor suppressor genes (Sun JX, et al: PLOS Comput Biol 14: e1005965, 2018). To evaluate performance of germline/somatic computational predictions in this series, we compared predictions against a subset of cases with available results from patient-matched germline testing or cell-free DNA (cfDNA) NGS that were performed as previously described (Clark TA, et al: J Mol Diagnostics 20:686-702, 2018; Khiabanian H, et al: JCO Precis Oncol 2:1-15, 2018); *BRCA1/2* genetic testing on a subset of patients at the Rutgers Cancer Institute of New Jersey were analyzed under the auspices of an IRB-approved protocol. For cfDNA analysis, somatic-like allele frequency (AF) was defined as mutations observed in cfDNA at 0%-20% AF. Germline-like AF was defined as mutations observed in cfDNA at 40%-60% AF (except for cfDNA samples with high circulating tumor DNA fraction [> 20%] that were excluded from the analysis as ambiguous). *BRCA1/2* alterations were categorized as biallelic, monoallelic, or unknown. Biallelic alterations were mutations with LOH of the wild-type allele, as determined by zygosity status (Sun JX, et al: PLOS Comput Biol 14: e1005965, 2018); homozygous deletion; or ≥ 2 *BRCA1* or ≥ 2 *BRCA2* alterations in a sample. Monoallelic alterations were heterozygous mutations (retained wild-type allele; Sun JX, et al: PLOS Comput Biol 14:e1005965, 2018). Alterations where zygosity status could not be determined were classified as unknown. Percent genome-wide LOH (gLOH) was calculated as a signature of HRD as previously described (Coleman RL, et al: Lancet 390:1949-1961, 2017; Swisher EM, et al: Lancet Oncol 18:75-87, 2017). In brief, LOH segments were inferred across the 22 autosomal chromosomes using the genome-wide aneuploidy/copy number profile and minor AFs of the > 3,500 polymorphic single nucleotide polymorphisms (SNPs) sequenced in the FoundationOne assay (Foundation Medicine, Cambridge, MA). Using a comparative genomic hybridization-like method, we obtained a log-ratio profile of the sample by normalizing the sequence coverage obtained at all exons and genome-wide SNPs against a process-matched normal control (Frampton GM, et al: Nat. Biotechnol 31: 1023-1031, 2013). This profile was segmented and interpreted using AFs of sequenced SNPs to estimate copy number (Ci) and minor allele count (Mi) at each segment (i). A segment was determined to have LOH if $Ci \neq 0$ and $Mi = 0$. Low tumor content or low aneuploidy were the most common reasons for failure to pass the quality control to perform gLOH inference. Two types of LOH segments were excluded from the calculation of percent gLOH: LOH segments that spanned ≥ 90% of a whole chromosome or chromosome arm because these LOH events usually arise through non-HRD mechanisms (eg, mitotic nondisjunction) and regions in which LOH inference was ambiguous. For each tumor, the percent gLOH was computed as 100× the total length of nonexcluded LOH regions (xi) divided by the total length of nonexcluded regions of the genome. An ultraviolet signature trinucleotide context was defined as the top-weighted alteration classes in COSMIC Signature 7 (A[C>T]C, C[C>T]A, C[C>T]C, C[C>T]G, C[C>T]T, G[C>T]C, G[C>T]T, T[C>T]A, T[C>T]C, T[C>T]G, T[C>T]T; https://cancer.sanger.ac.uk/cosmic/signatures_v2; Alexandrov LB, et al: Nature 500:415-421, 2013). Tumor mutational burden was calculated by counting the number of synonymous and nonsynonymous mutations across a 0.8- to 1.2-Mb region, with computational germline status filtering, and reporting the result as mutations/Mb; this method has been previously validated for accuracy against whole-exome sequencing (Chalmers ZR, et al: Genome Med 9:34, 2017).

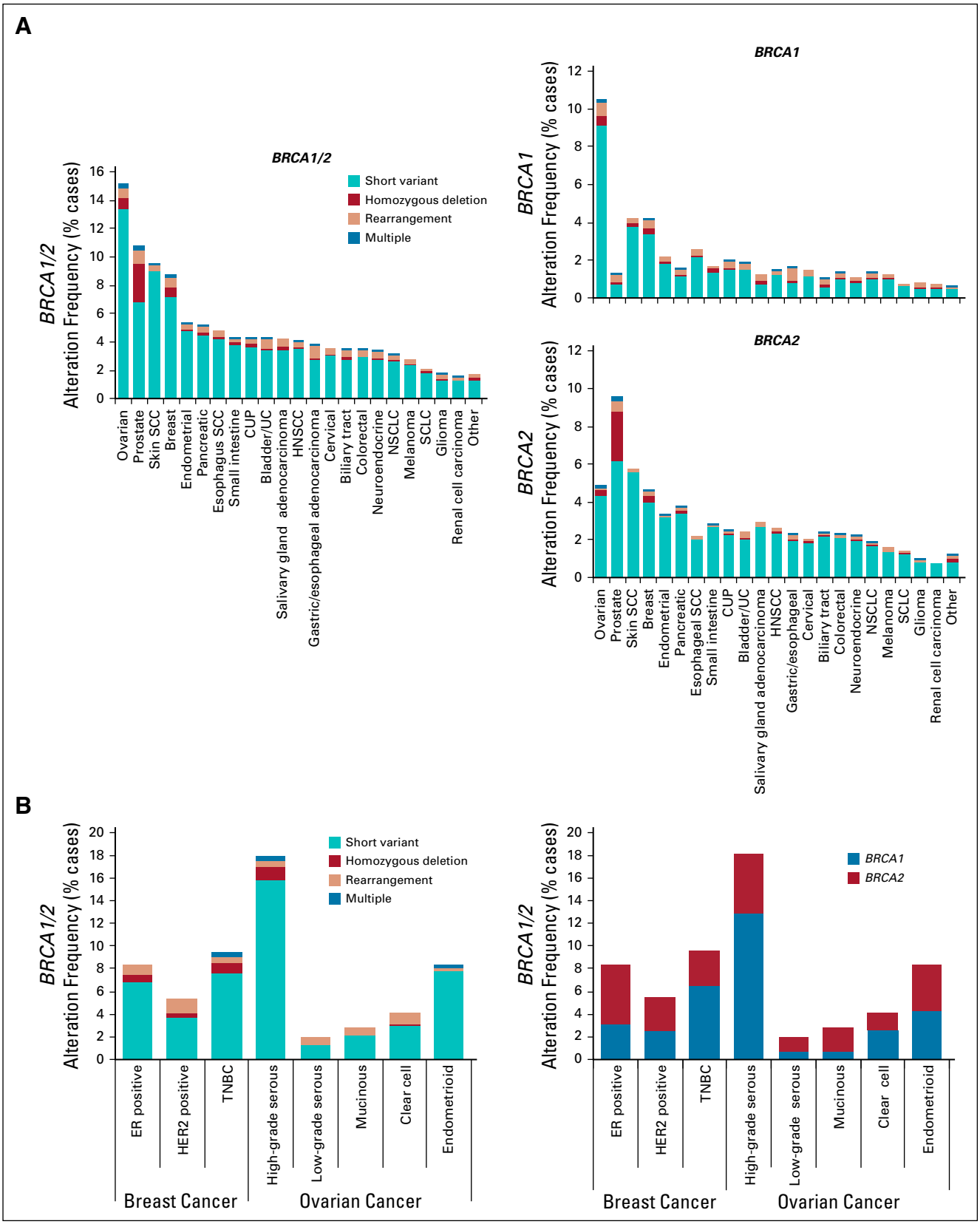


FIG A1. (A) Frequency of *BRCA1* and *BRCA2* alterations across multiple cancer types. Multiple indicates samples with two or more concurrent *BRCA* alteration types. (B) Frequency of *BRCA1/2* alterations in the subset of ovarian and breast cancer cases where molecular/histologic subtype information was available. For breast cancer, estrogen receptor (ER) status was available for a subset of samples; human epidermal (continued on following page)

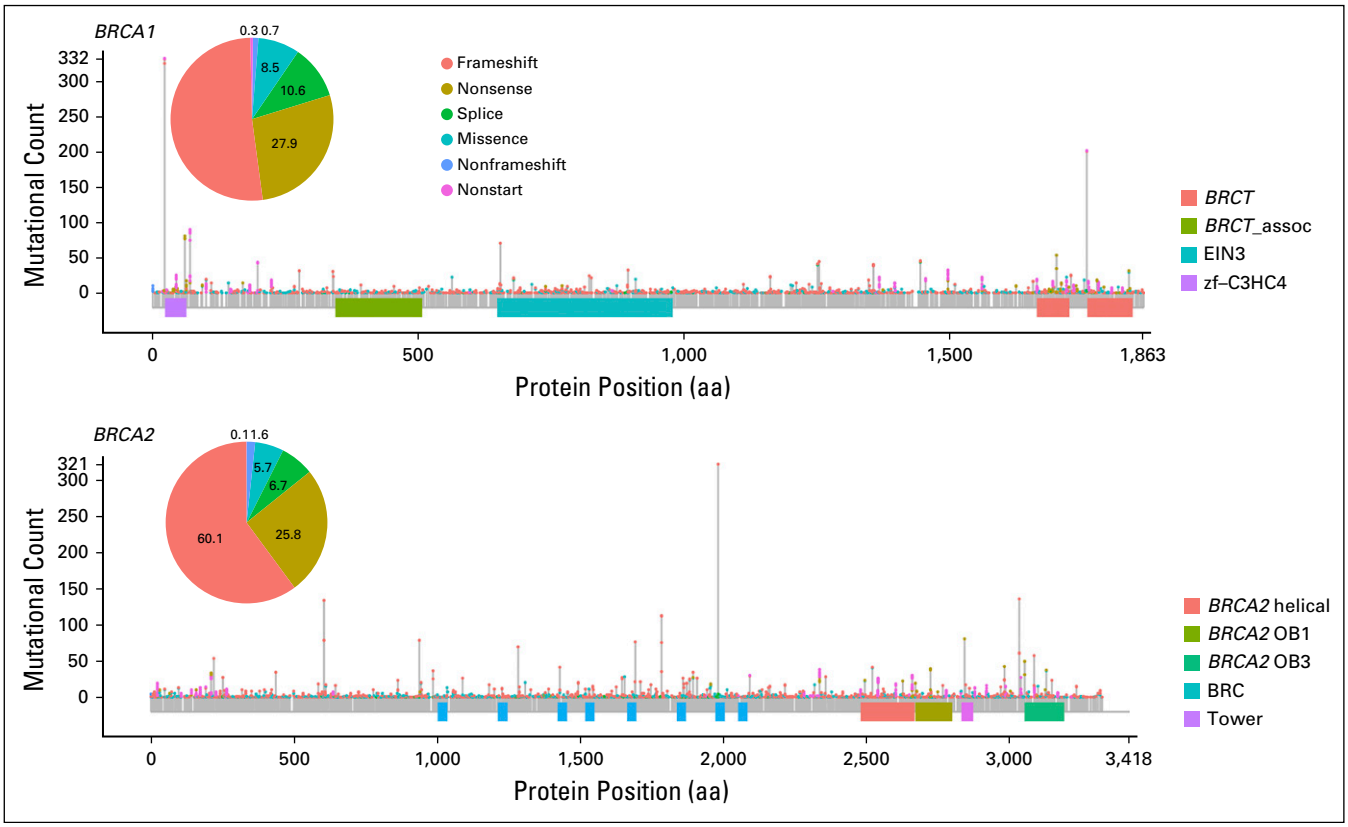


FIG A2. Summary of all *BRCA1* and *BRCA2* mutations included in the study by location and type. aa, amino acid.

FIG A1. (Continued). growth factor receptor 2 (HER2) status was determined on the basis of the presence or absence of a copy number amplification; triple-negative breast cancer (TNBC) was defined as ER-negative, HER2-negative samples. CUP, cancer of unknown primary; HNSCC, head and neck squamous cell carcinoma; NSCLC, non–small-cell lung cancer; SCC, squamous cell carcinoma; SCLC, small-cell lung cancer; UC, urothelial cancer.

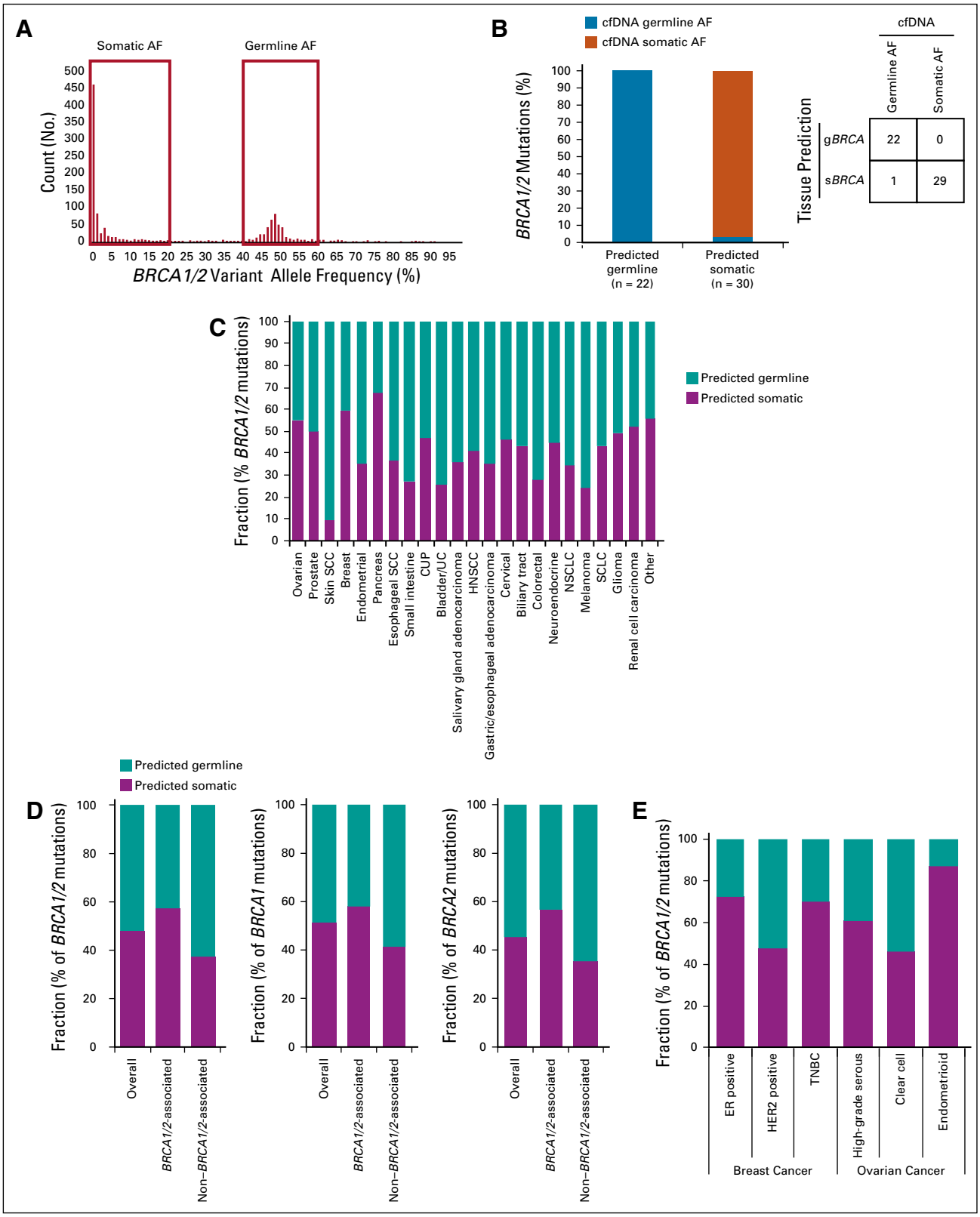


FIG A3. (A and B) Comparison of tissue-based germline/somatic predictions to *BRCA1/2* allele frequencies (AFs) in patient-matched cell-free DNA (cfDNA). (A) Distribution of AFs for 1,207 cfDNA samples with *BRCA1/2* mutation. Mutations with somatic AFs were defined as those identified in cfDNA at 0%-20% AF. Mutations with germline AF were defined as those identified in cfDNA at 40%-60% AF, except for cases with high circulating tumor DNA fraction (20%) that were excluded from the analysis as ambiguous AF. (B) Fifty-two tissue-derived germline *BRCA1/2* (g*BRCA1/2*) and somatic *BRCA1/2* (s*BRCA1/2*) mutation predictions (from 48 tissue samples) were compared with patient-matched (continued on following page)

FIG A3. (Continued). cfDNA AFs; 100.0% of germline predictions (22 of 22) were observed at germline-like AF and 96.7% of somatic predictions (29 of 30) were observed at somatic-like AF. (C-E) Predicted germline/somatic status calls were made for each *BRCA1* or *BRCA2* short variant mutation. For mutations yielding a successful call, frequency of predicted germline *v* somatic mutation was determined for (C) *BRCA1/2* across cancer types, (D) *BRCA1/2* (grouped and individually) overall (n = 5,845) for *BRCA1/2*-associated cancers (breast, ovarian, pancreatic, prostate; n = 3,061) and non–*BRCA1/2*-associated cancers (n = 2,784), and (E) *BRCA1/2* for the subset of ovarian and breast cancer cases where molecular/histologic subtype information was available. CUP, cancer of unknown primary; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; HNSCC, head and neck squamous cell carcinoma; NSCLC, non–small-cell lung cancer; SCC, squamous cell carcinoma; SCLC, small-cell lung cancer; TNBC, triple-negative breast cancer; UC, urothelial cancer.

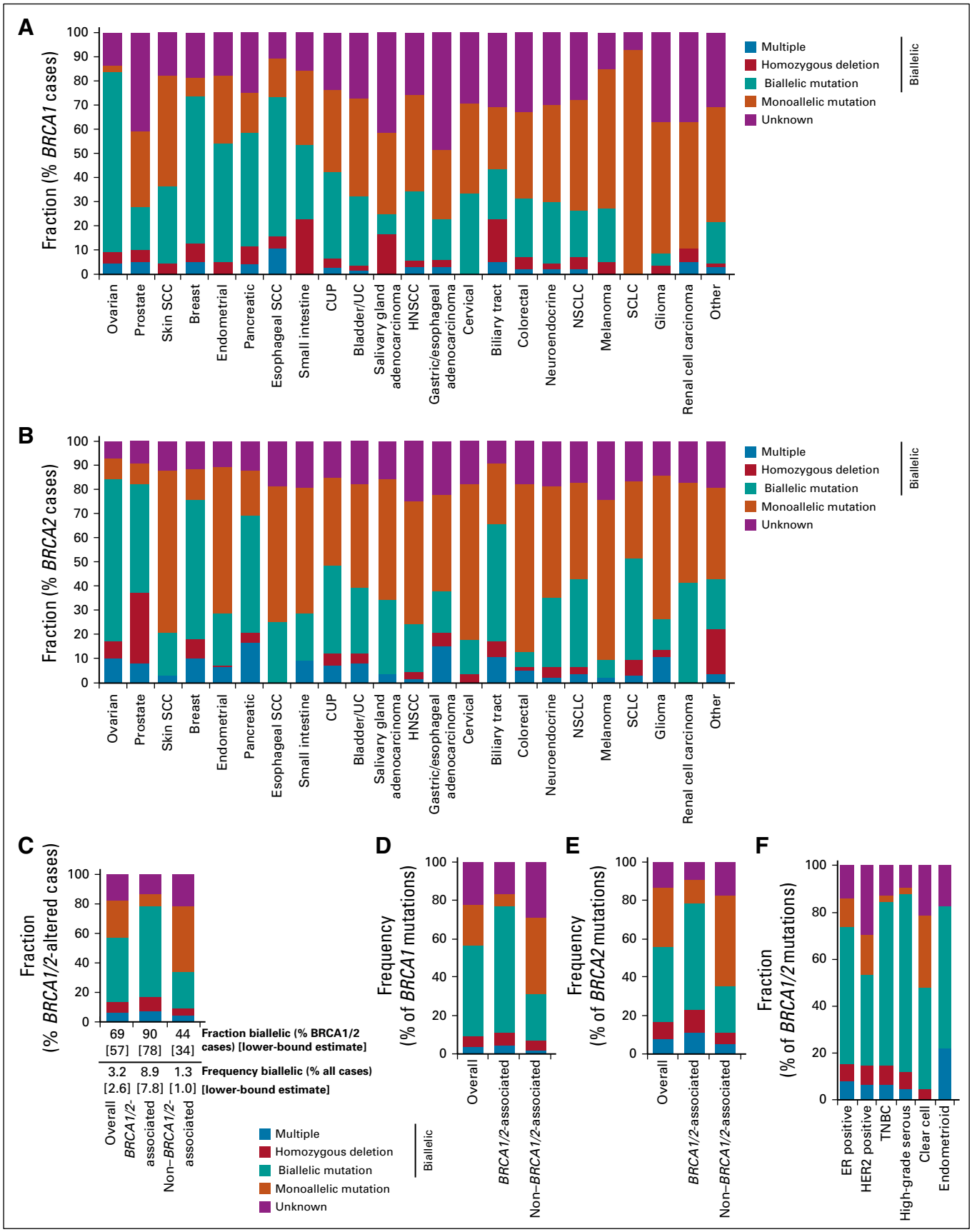


FIG A4. Relative fraction of *BRCA1/2*-altered cases with biallelic or monoallelic alteration was determined for (A) *BRCA1* across cancer types and (B) *BRCA2* across cancer types. (C) *BRCA1/2*; (D) *BRCA1*; (E) *BRCA2* for overall, *BRCA1/2*-associated cancers, and non–*BRCA1/2*-associated cancers; and (F) *BRCA1/2* for the subset of ovarian and breast cancer cases where molecular/histologic subtype information was available. CUP, cancer of unknown primary; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; HNSCC, head and neck squamous cell carcinoma; NSCLC, non–small-cell lung cancer; SCC, squamous cell carcinoma; SCLC, small-cell lung cancer; TNBC, triple-negative breast cancer; UC, urothelial cancer.

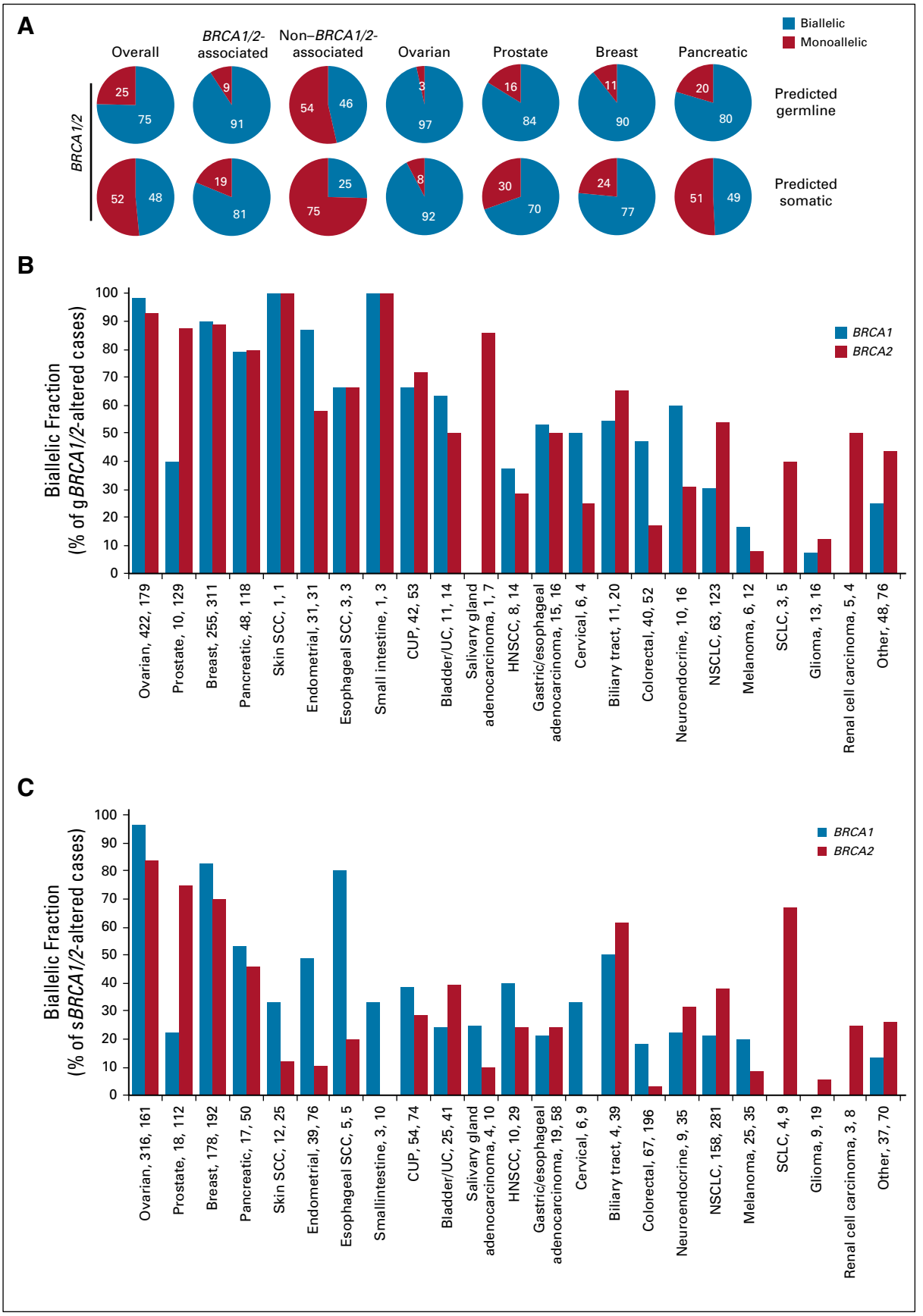
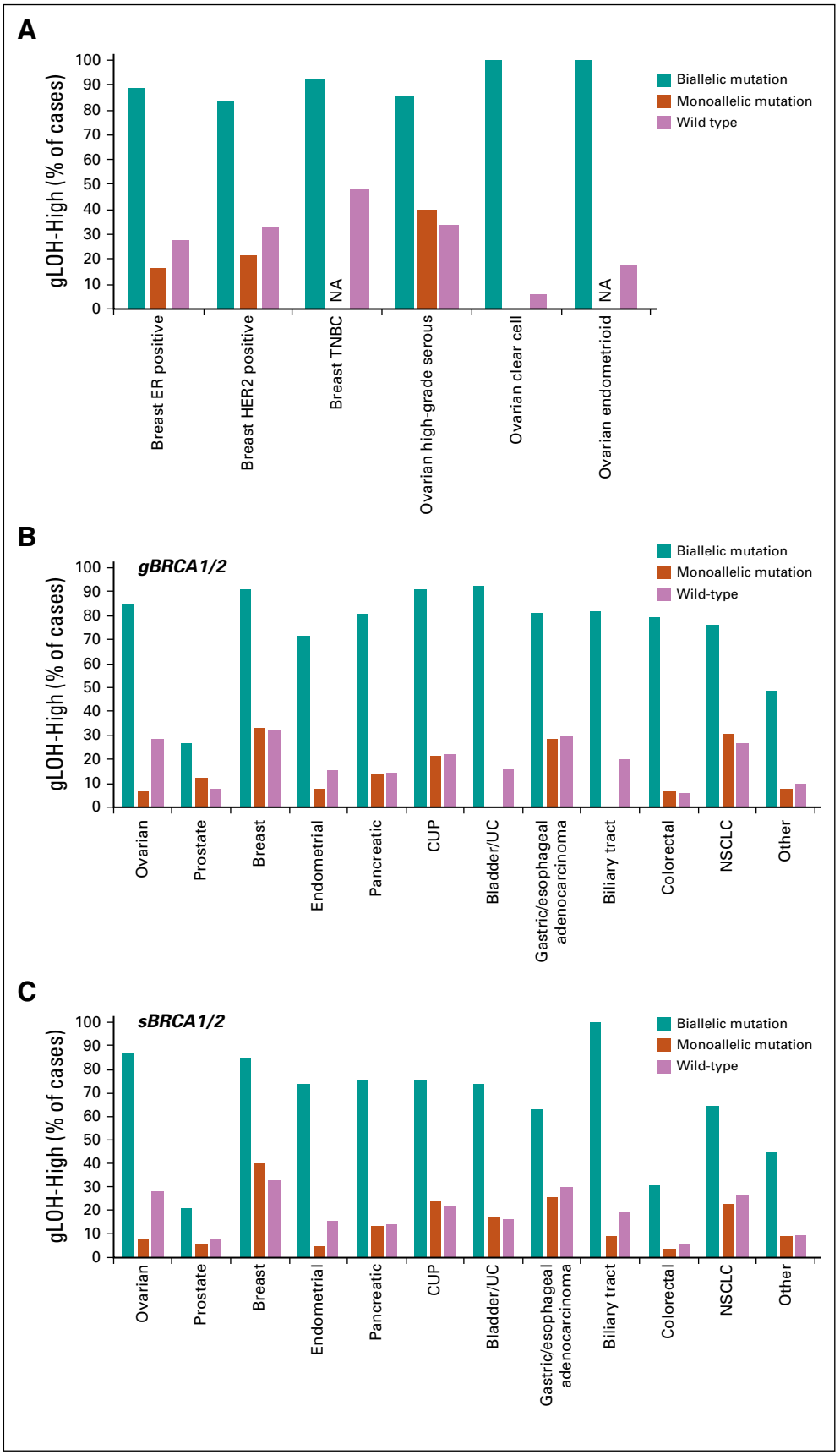


FIG A5. (A) *BRCA1/2* mutations (grouped) with a germline (g*BRCA1/2*) or somatic (s*BRCA1/2*) prediction were evaluated for biallelic/monoallelic status for all cancers, *BRCA1/2*-associated cancers (as a group and as individual cancer types), and non–*BRCA1/2*-associated cancers (see Data Supplement). Biallelic fraction was assessed for (B) g*BRCA1/2*- and (C) s*BRCA1/2*-mutated cases. Numbers on the *x*-axis indicate number of cases assessed for biallelic status g*BRCA1* or g*BRCA2*. CUP, cancer of unknown primary; HNSCC, head and neck squamous cell carcinoma; NSCLC, non–small-cell lung cancer; SCC, squamous cell carcinoma; SCLC, small-cell lung cancer; UC, urothelial cancer.



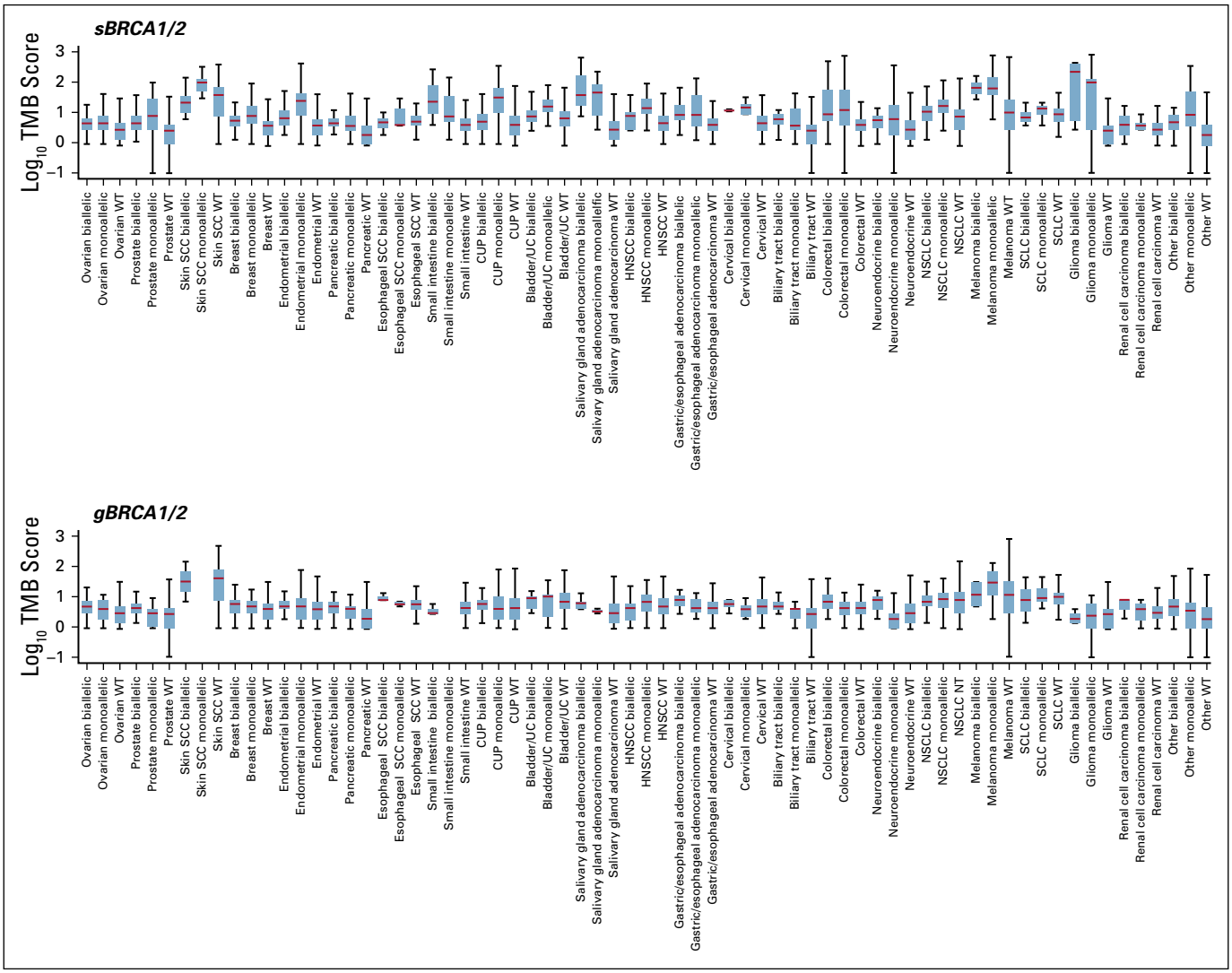


FIG A7. Cases with somatic *BRCA1/2* (s*BRCA1/2*) or germline *BRCA1/2* (g*BRCA1/2*) biallelic mutation, monoallelic mutation, or wild-type (WT) *BRCA1/2* were plotted against tumor mutational burden (TMB in mutations/Mb; $\log_{10}$ score). Box and whisker plot where the box spans the first and third quartiles, the median is denoted by the horizontal line in the box, and whiskers indicate maximum and minimum values within 1.5× the interquartile range (see Data Supplement). CUP, cancer of unknown primary; HNSCC, head and neck squamous cell carcinoma; NSCLC, non–small-cell lung cancer; SCC, squamous cell carcinoma; SCLC, small-cell lung cancer; UC, urothelial cancer.

FIG A6. (A) The frequency of *BRCA1/2* biallelic, monoallelic, and wild-type cases that were high genome-wide loss of heterozygosity (gLOH-high) was compared in the subset of ovarian and breast cancer cases where molecular/histologic subtype information was available (see Data Supplement). (B) Frequency of predicted germline *BRCA1/2* (g*BRCA1/2*) biallelic, monoallelic, and wild-type cases that were gLOH-high (see Data Supplement). (C) Frequency of predicted somatic *BRCA1/2* (s*BRCA1/2*) biallelic, monoallelic, and wild-type cases that were gLOH-high (see Data Supplement). CUP, cancer of unknown primary; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; NA, not available (no assessable alterations); NSCLC, non–small-cell lung cancer; TNBC, triple-negative breast cancer; UC, urothelial cancer.