Influence of Germline \textit{BRCA} Genotype on the Survival of Patients with Triple-Negative Breast Cancer

Cynthia Villarreal-Garza\textsuperscript{1,2}, Ana S. Ferrigno\textsuperscript{1}, Alejandro Aranda-Gutierrez\textsuperscript{3}, Paul H. Frankel\textsuperscript{4}, Nora H. Ruel\textsuperscript{1}, Alan Fonseca\textsuperscript{2}, Steven Narod\textsuperscript{1}, Yanin Chavarri-Guerra\textsuperscript{3}, Erika Sifuentes\textsuperscript{2}, Maria Cristina Magallanes-Hoyos\textsuperscript{2}, Josef Herzog\textsuperscript{4}, Danielle Castillo\textsuperscript{4}, Rosa M. Alvarez-Gomez\textsuperscript{2}, Alejandro Mohar-Betancourt\textsuperscript{4}, and Jeffrey N. Weitzel\textsuperscript{6}

\textbf{ABSTRACT}

The presence of \textit{BRCA} pathogenic variants (PV) in triple-negative breast cancer (TNBC) is associated with a distinctive genomic profile that makes the tumor particularly susceptible to DNA-damaging treatments. However, patients with \textit{BRCA} PVs can develop treatment resistance through the appearance of reversion mutations and restored \textit{BRCA} expression. As copy-number variants (CNV) could be less susceptible to reversion mutations than point mutations, we hypothesize that carriers of \textit{BRCA} CNVs may have improved survival after treatment compared with carriers of other \textit{BRCA} PVs or \textit{BRCA} wild-type. Women diagnosed with stage I–III TNBC at \textless 50 years at a cancer center in Mexico City were screened for \textit{BRCA} PVs using a recurrent PV assay (HISPANEL; 77% sensitivity). Recurrence-free survival (RFS) and overall survival (OS) were compared according to the mutational status. Among 180 women, 17 (9%) were carriers of \textit{BRCA1} ex9–12del CNVs and 26 (14%) of other \textit{BRCA} PVs. RFS at ten years for the whole cohort was 79.2\% [95% confidence interval (CI), 72.3–84.6], with no significant differences according to mutational status. 10-year OS for the entire cohort was 85.3\% (95% CI, 78.7–90.0), with \textit{BRCA} CNV carriers demonstrating numerically superior OS rates other PV carriers and non-carriers (100\% vs. 78.6\% and 84.7\%; log-rank \( P = 0.037 \) and \( P = 0.051 \), respectively). This study suggests that \textit{BRCA1} ex9–12del CNV carriers with TNBC may have a better OS, and supports the hypothesis that the genotype of \textit{BRCA} PVs may influence survival by limiting treatment resistance mediated by reversion mutations among CNV carriers.

\textbf{Significance:} Large CNV \textit{BRCA} carriers in a cohort of young Mexican patients with TNBC had superior OS rates than carriers of other \textit{BRCA} pathogenic variants (i.e., small indels or point mutations). We hypothesize that this is due to the resistance of CNVs to reversion mutations mediating resistance to therapy. If validated, these findings have important prognostic and clinical treatment implications for \textit{BRCA}-associated breast cancers.

\textbf{Introduction}

Breast cancer is the most frequently diagnosed malignancy and the leading cause of cancer-related death in females (1). It is estimated that 5\% to 10\% of breast cancer cases are related to inherited predisposition genes, with germline \textit{BRCA1} and \textit{BRCA2} pathogenic variants (\textit{BRCA PV}) being the most common (2, 3). The presence of these germline PVs can have an important effect on the characteristics of breast tumors. \textit{BRCA1}-mutated breast cancers tend to have a high histologic grade and be classified as triple-negative breast cancer (TNBC), while tumors associated with \textit{BRCA2} PVs are associated with estrogen receptor (ER)-positive and HER2-negative subtype, but with a tendency toward a higher proportion of TNBC with increasing age (4).

Despite the effects of \textit{BRCA} PVs on the clinicopathologic features of breast tumors, the prognostic effect of these PVs is less clear (5–7). In general, it appears that no significant difference exists in overall survival (OS) in comparison to phenotypically similar tumors of noncarriers of \textit{BRCA} PVs, except for a tendency toward a worse OS in ER-positive \textit{BRCA}-associated tumors (8, 9). Recent studies of TNBC did not reveal significant correlation between \textit{BRCA} status and recurrence-free survival (RFS) or OS (10–12). Nevertheless, the Prospective Outcomes in Sporadic versus Hereditary breast cancer (POS-H) study reported that \textit{BRCA} PV carriers with young-onset TNBC (defined as women aged \textless 40 years at diagnosis) may have a survival advantage in the first 2 years after detection, although this was not statistically significant after 5 years (12). A possible
A key function of the BRCA tumor suppressor genes is to maintain genomic stability through homologous recombination repair (HRR) of DNA double-strand breaks. Hence, BRCA-deficient cases have a distinctive genomic aberration profile that makes them particularly susceptible to certain treatments, such as platinum-based chemotherapy regimens and PARP inhibitors (PARPi; refs. 3, 15). However, patients with BRCA-PV–associated breast cancer can develop resistance to DNA-damaging therapies through the appearance of reversion mutations, which restore the reading frame and can recover at least partial BRCA function (16, 17). Large genomic rearrangements, referred hereafter as copy-number variants (CNV), account for 10% to 40% of germline BRCA1 and 3% of germline BRCA2 PVs (18, 19). Pathogenic small insertion/deletion or base substitution variants that cause early protein truncation and CNVs both cause the loss of critical functional domains of BRCA proteins (20). However, with large segments of the gene missing, we presume that CNVs are less susceptible to reversion mutations. Thus, we hypothesize that carriers of BRCA CNVs with TNBC may have an improved survival after treatment compared with carriers of other BRCA PVs secondary to the inability of restoring BRCA expression.

We previously described a young Mexican TNBC cohort where the BRCA1 ex9–12del CNV (also known as the Mexican BRCA1 founder mutation) represented 40% of all germline BRCA PVs (21). Of note, TNBC represents a substantial proportion of breast cancer cases diagnosed in Mexico (16%–23%; refs. 22, 23), and it is particularly prevalent in cases diagnosed at a young age (<50 years) and in those with germline BRCA PVs (4). The aim of this study is to compare the survival of young patients with TNBC with the BRCA1 ex9–12del CNV, other BRCA PVs (small insertion/deletion or base substitution variants), and noncarriers to explore the influence of the BRCA genotype on survival.

Materials and Methods

Patient Cohort

A cohort of Mexican women diagnosed with TNBC at 50 years of age or younger between January 2006 and January 2012 that received treatment at the National Cancer Institute (INCan) in Mexico City has been described previously (21). To be eligible for this analysis, ER- and PR-negative status (<1% nuclear staining by IHC) and HER2 overexpression status (0 or 1+ by IHC or 2+ by IHC but demonstrated not to be amplified with FISH) was ascertained from pathology reports of tumor tissue. The patients provided written informed consent in accordance with recognized ethical guidelines (Declaration of Helsinki; U.S. Common rule), and the studies were approved by the INCan institutional review board.

Screening for BRCA Germline PVs

As described previously (21), a total of 190 patients were analyzed for BRCA germline PVs using the HISPEL PANEL assay, which screens for 114 indels and point BRCA PVs described as prevalent in Hispanic patients with breast cancer by five multiplex reactions on the Sequenom® (San Diego, CA) MassARRAY platform (matrix-assisted laser desorption/ionization—time-of-flight mass spectrometry) and includes a three-primer PCR assay for the BRCA1 ex9–12del CNV (20).

Clinicopathologic Data Collection

Electronic medical records from INCan’s digital database were retrospectively reviewed up to December 31, 2019. The data collected for the 190 BRCA-characterized patients included patient age at diagnosis, clinical and/or pathologic TNM staging (according to the 7th edition of the American Joint Committee on Cancer Staging System), chemotherapy regimens used, histologic grade, mutational status, disease recurrence, secondary primary malignancies, and vital status. OS was defined as the time elapsed from histologic diagnosis of breast cancer until death by any cause and RFS was defined as the time elapsed from diagnosis until disease recurrence or death by any cause.

As the objective of this study was to compare OS and RFS rates between carriers of BRCA1 ex9–12del, carriers of other BRCA PVs, and patients without a detectable BRCA PV, patients with in situ or stage IV disease were excluded from this analysis. Ultimately, 10 patients were excluded from this study: unspecified disease stage (n = 2), in situ disease (n = 1), and stage IV at diagnosis (n = 7), resulting in a total of 180 patients with BRCA-characterized TNBC included in the statistical analysis (Supplementary Fig. S1).

Statistical Analysis

Statistical analyses were carried out using STATA version 13.0 software (StataCorp), R 4.0.2 (R Core Team 2020), and SAS9.4 packages. The patients were grouped according to BRCA mutational status. Descriptive statistics were undertaken using frequency and proportions for categorical variables and median and range for quantitative variables. Mann–Whitney U, χ², and Fisher exact tests were used for exploring differences according to group category, as appropriate. OS and RFS were calculated using the Kaplan–Meier method. The log-rank test was used for survival comparisons between groups, and the exact calculation method was applied for comparisons involving zero events. Cox regression analyses were carried out to estimate the survival HRs associated with the class of BRCA germline PV. Noting that HR is undefined with zero events in one group, we reported survival and a confidence interval (CI) at a fixed time (e.g., 10 years), although with statistics from the log-rank test. The Beta Product Confidence Procedure (BPCP) was used to calculate the confidence intervals at specific timepoints if no survival failure events were observed in a group category. The BPCP method provides conservative confidence bounds, despite limited sample size. Statistical significance was set at a two-sided P value of < 0.05. No adjustment for multiple hypothesis testing was performed in the context of this retrospective analysis.

Results

Patient Demographics and Clinical Characteristics

A total of 180 patients with TNBC aged <50 years at diagnosis were included in this study, of which 43 patients (24%) were BRCA PV carriers. Of these, 17 (40%) were found to harbor the BRCA1 ex9–12del CNV, while 26 (60%) had other BRCA PVs (Table 1). The median follow-up from breast cancer diagnosis for the entire cohort was 10.3 years (95% CI, 9.5–10.8). As shown in Supplementary Table S1, no statistical differences of median follow-up were found according to mutational status (median follow-up of 10.7 years for BRCA CNV carriers, 10.1 years for other BRCA PV carriers, and 10.3 years for noncarriers).

Clinical characteristics are summarized in Table 2. BRCA PV carriers were diagnosed with breast cancer at a younger age than noncarriers (38 vs. 43 years; P < 0.001), with no observed difference between carriers of CNVs and other
TABLE 1  BRCA PVs identified in this cohort of patients with TNBC.

| Gene  | Exon(s) | BIC variant | HGVS variant | n     |
|-------|---------|-------------|--------------|-------|
| BRCA1 | 9–12    | ex9–12del   | c.548_?_4185+?del | 17    |
| BRCA1 | 11      | 948ins10    | c.815_824dup  | 5     |
| BRCA1 | 5       | 330A>G (R71G) | c.211A>G     | 4     |
| BRCA1 | 11      | 2925del14   | c.2806_2809del | 4     |
| BRCA1 | 13      | 4446C>T (R1443X) | c.4327C>T  | 3     |
| BRCA1 | 2       | 185delAG    | c.66_67del   | 2     |
| BRCA1 | 11      | 387delTA    | c.3759_3760del | 2     |
| BRCA1 | 11      | 3717C>T (Q1200X) | c.3598C>T | 1     |
| BRCA1 | 11      | 2415delAG   | c.2292_2295del | 1     |
| BRCA1 | 18      | 5242C>A (A1707E) | c.5123C>A  | 1     |
| BRCA1 | 11      | 1979delT    | c.1860del   | 1     |
| BRCA1 | ?       | IVS20+1delG | c.5277+1del  | 1     |
| BRCA2 | 11      | 2452C>T (Q742X) | c.2224C>T  | 1     |

Abbreviations: BIC, Breast Cancer Information Core; HGVS, Human Genome Variation Society.

PVs. There were no significant differences observed between groups with respect to clinical stage or histologic grade. However, BRCACNV carriers had a tendency toward negative lymph node status at diagnosis (10/16 (62.5%) CNV carriers with negative lymph node involvement vs. 9/25 (36%) other PV carriers; vs. 43/136 (32%) noncarriers; three-group exact χ² test P = 0.049). BRCA PV carriers had a higher prevalence of bilateral breast cancer compared with noncarriers (19% vs. 4%; P = 0.003), with a nonsignificant excess of bilateral breast cancer in BRCA CNV carriers compared with the other PV group (24% vs. 15%; P = 0.692).

Regarding general treatment strategies, no statistically significant differences were observed according to mutational status. Overall, 47% were treated with platinum-based chemotherapy regimens and none were treated with PARPi.

A total of 21 patients presented with a second primary malignancy during the follow-up period. In the BRCA CNV carrier group, second primaries were identified in the breast, ovaries, and bladder, while BRCA point mutation carriers were diagnosed with second primaries only in the contralateral breast and ovaries (Supplementary Table S2). BRCA PV carriers experienced a higher rate of second primary malignancies than noncarriers (36% vs. 7%; P = 0.002), with patients in the BRCA CNV group demonstrating a greater tendency, albeit nonsignificant, toward being diagnosed with a second cancer compared with other PV carriers (41% vs. 15%; P = 0.080).

Survival Estimates

A total of 28 patients died during the follow-up period. The absence of deaths in the BRCA CNV group during the follow-up period is noteworthy. In contrast, 19% of carriers of other BRCA PVs and 17% of noncarriers experienced death. Of note, all deaths documented were attributed to the primary breast malignancy. Survival rates at 5 and 10 years are shown in Table 3.

OS was 86.3% (95% CI, 80.0–90.7) for the whole cohort at ten years after diagnosis of TNBC. Overall, no statistically significant differences were observed in OS between BRCA PV carriers and noncarriers (HR, 0.66; 95% CI, 0.25–1.74). However, when comparing BRCA CNV carriers versus carriers of other BRCA PVs (Fig. 1A and B), OS was significantly higher in the former (100% vs. 78.6% at 10 years; log-rank P value of the Kaplan–Meier model = 0.037). OS rates of BRCA CNVs were also numerically superior to noncarriers, although not reaching statistical significance (100% vs. 84.7% at 10 years; log-rank P = 0.06).

The survival advantage observed in the carriers of BRCA CNVs was maintained after exclusion of the one patient in the group of carriers of other BRCA PVs that had a PV in BRCA2 (100% vs. 77.5% at 10 years; two-sided log-rank P value of the Kaplan–Meier model = 0.030). Of note, node-negative patients had 0 of 10 registered deaths in CNV group versus 1 of 9 in other PV group, while node-positive patients had 0 of 6 deaths documented in CNV group and 4 of 16 deaths in the other PV group.

In total, 34 patients experienced disease recurrence. As shown in Table 2, all recurrences documented in BRCA PV carriers and the majority of those experienced by noncarriers were classified as distant disease. Overall, the RFS rate for the whole cohort was 79.2% (95% CI, 72.3–84.6) at ten years after diagnosis of TNBC. No statistically significant differences were observed in RFS between groups (Table 3; Fig. 1C and D).

Discussion

In this study, we explored the survival outcomes of a cohort of BRCA-characterized women with TNBC aged ≤50 years at diagnosis. Notably, OS rates did not differ significantly between carriers of BRCA PVs and noncarriers. However, when examining subgroups according to BRCA variant type, a survival advantage was observed in the BRCA CNV carrier group. Specifically, the carriers of the BRCA1 ex9–12del CNV in our cohort had a 100% OS rate at 10 years, which was numerically superior to the 10-year OS observed among carriers of other BRCA PVs and noncarriers (78.6% and 84.7%, respectively). We believe that the findings of our study have important clinical prognostic and treatment implications for patients with breast cancer who carry a BRCA PV as they suggest that genotype can exert a differential impact on survival. Further studies confirming the observed difference are needed.

The Mexican BRCA1 founder CNV (BRCA1 ex9–12del) is one of the most frequently reported population-specific CNVs (24). It is characterized by a deletion of 14.7 kilobases, which results in the loss of multiple functional protein domains and premature BRCA truncation. Specifically, the BRCA1 ex9–12del CNV results in the complete loss of the p53, pRb, Rad50, Rad51, NLS1, and NLS2 interacting domains, and partial loss of the ERα domain (20). This aberration was first reported in 2007 after it was detected in 3.8% of unrelated Hispanic American families that had a personal history of breast cancer or ovarian cancer (20). In our referral center, it has been reported that this PV accounts for 35% of BRCA-associated ovarian cancer cases and 29% of BRCA-associated breast cancer cases (24). The remarkable frequency of the Mexican BRCA1 founder mutation, particularly in women originating from Central and Southern Mexico, constitutes a regional public health problem. Given its high prevalence in our setting, we employed the BRCA1 ex9–12del genotype to analyze if CNVs have a different prognostic impact than point mutations in the same gene.

Previous studies have raised the possibility of BRCA PVs having a different prognostic impact based on factors other than their germline presence. For example, a meta-analysis by Xie and colleagues showed that, although the presence of BRCA1 PVs had no correlation with prognosis in patients with breast cancer, BRCA1 promoter methylation was associated with worse survival.
TABLE 2  Select clinicopathologic characteristics of included TNBC cases.

|                  | All BRCA PV carriers | BRCA CNV carriers<sup>a</sup> | Other BRCA PVs<sup>b</sup> | P<sup>c</sup> | Noncarriers | P<sup>d</sup> |
|------------------|----------------------|-------------------------------|---------------------------|-------------|-------------|-------------|
| n                | 43                   | 17                            | 26                        | —           | 137         | —           |
| Age at diagnosis |                      |                               |                           |             |             |             |
| Median (range)   | 38 (23–50)           | 39 (28–50)                    | 37.5 (23–48)              | 0.784       | 43 (25–50)  | <0.001      |
| Missing          | 0                    | 0                             | —                         |             | 1 (1%)      |             |
| Bilateral BC     | 8 (19%)              | 4 (24%)                       | 4 (15%)                   | 0.692       | 5 (4%)      | 0.003       |
| Stage            |                      |                               |                           |             |             |             |
| I                | 5 (12%)              | 2 (12%)                       | 3 (12%)                   | 0.326       | 8 (6%)      | 0.277       |
| II               | 22 (51%)             | 11 (65%)                      | 11 (42%)                  |             | 63 (46%)    |             |
| III              | 16 (37%)             | 4 (24%)                       | 12 (46%)                  |             | 66 (48%)    |             |
| Nodal status     |                      |                               |                           |             |             |             |
| Positive         | 22 (51%)             | 6 (35%)                       | 16 (62%)                  | 0.120       | 93 (68%)    | 0.095       |
| Negative         | 19 (44%)             | 10 (59%)                      | 9 (35%)                   |             | 43 (31%)    |             |
| Missing          | 2 (5%)               | 1 (6%)                        | 1 (4%)                    | —           | 1 (1%)      | —           |
| Histologic grade |                      |                               |                           |             |             |             |
| 1                | 0                    | 0                             | 0                         | >0.999      | 4 (3%)      | 0.286       |
| 2                | 2 (5%)               | 1 (6%)                        | 1 (4%)                    | 13 (10%)    |             |             |
| 3                | 40 (93%)             | 16 (94%)                      | 24 (92%)                  |             | 114 (83%)   |             |
| Missing          | 1 (2%)               | 0                             | 1 (4%)                    | —           | 6 (4%)      |             |
| Mastectomy       |                      |                               |                           |             |             |             |
| Total            | 39 (91%)             | 16 (94%)                      | 23 (89%)                  | 0.691       | 117 (85%)   | 0.662       |
| Partial          | 3 (7%)               | 1 (6%)                        | 2 (8%)                    | 14 (10%)    |             |             |
| Not performed    | 1 (2%)               | 0                             | 1 (4%)                    | 6 (4%)      |             |             |
| Chemotherapy     |                      |                               |                           |             |             |             |
| Neoadjuvant      | 17 (40%)             | 7 (41%)                       | 10 (39%)                  | 0.947       | 67 (49%)    | 0.602       |
| Adjuvant         | 17 (40%)             | 6 (35%)                       | 11 (42%)                  | 46 (34%)    |             |             |
| Both             | 9 (21%)              | 4 (24%)                       | 5 (19%)                   | 21 (15%)    |             |             |
| None             | 0                    | 0                             | 0                         | 1 (1%)      |             |             |
| Missing          | 0                    | 0                             | —                         | —           | 2 (2%)      | —           |
| Pathologic complete response<sup>g</sup> |                    |                               |                           |             |             |             |
| Yes              | 15 (58%)             | 7 (64%)                       | 8 (53%)                   | >0.999      | 39 (44%)    | 0.362       |
| No               | 10 (39%)             | 4 (36%)                       | 6 (40%)                   | 44 (50%)    |             |             |
| Missing          | 1 (4%)               | 0                             | 1 (7%)                    | —           | 5 (6%)      | —           |
| Treatment with platinum-based chemotherapy |                |                               |                           |             |             |             |
| Yes              | 23 (53%)             | 7 (41%)                       | 16 (62%)                  | 0.225       | 62 (45%)    | 0.484       |
| No               | 20 (47%)             | 10 (59%)                      | 10 (38%)                  | 72 (53%)    |             |             |
| Missing          | 0                    | 0                             | 0                         | —           | 3 (2%)      | —           |
| Radiotherapy     |                      |                               |                           |             |             |             |
| Yes              | 30 (70%)             | 12 (71%)                      | 18 (69%)                  | >0.999      | 101 (74%)   | 0.695       |
| No               | 13 (30%)             | 5 (23%)                       | 5 (23%)                   | 36 (26%)    |             |             |
| Risk-reducing surgeries |            |                               |                           |             |             |             |
| Contralateral mastectomy<sup>f</sup> | 14 (33%) | 5 (29%) | 9 (35%) | >0.999 | 2 (2%) | <0.001 |
| Salpingo-oophorectomy | 17 (40%) | 8 (47%) | 9 (35%) | 0.528 | 0 | <0.001 |

(Continued on the following page)
TABLE 2 Select clinicopathologic characteristics of included TNBC cases. (Cont’d)

| BRCA mutation carriers | All BRCA PV carriers | BRCA CNV carriers | Other BRCA PVs | \( P^c \) | Noncarriers | \( P^d \) |
|------------------------|----------------------|-------------------|----------------|-----------|-------------|----------|
| Second primary malignancy |                      |                   |                |           |             |          |
| Any site               | 11 (36%)             | 7 (41%)           | 4 (15%)        | 0.080     | 10 (7%)     | 0.002    |
| Breast                 | 7 (64%)              | 4 (57%)           | 3 (75%)        | —         | 5 (50%)     | —        |
| Ovarian                | 3 (27%)              | 2 (29%)           | 1 (25%)        | —         | 1 (10%)     | —        |
| Thyroid                | 0                    | 0                 | 0              | —         | 2 (20%)     | —        |
| Other\(^g\)            | 1 (9%)               | 1 (14%)           | 0              | —         | 2 (20%)     | —        |

Abbreviation: BC, breast cancer.

\(^a\)All BRCA CNV were BRCA1 ex9–12del (Mexican founder mutation).

\(^b\)An indel/point PV was identified in BRCA1 in 25 patients and in BRCA2 in one patient.

\(^c\)P value comparing BRCA CNV carriers versus other BRCA PVs.

\(^d\)P value comparing All BRCA PV carriers versus noncarriers.

\(^e\)Only patients that received neoadjuvant treatment. Missing values are patients who did not undergo surgery (refused the procedure or died before the surgery was performed).

\(^f\)Analysis restricted to contralateral mastectomy in patients without bilateral breast cancer (wherein the procedure is therapeutic).

\(^g\)Other sites of second primary malignancies were cervical in situ cancer, vulvar in situ cancer, and Hodgkin lymphoma diagnosed in a single noncarrier of BRCA PVs, chondrosarcoma in another noncarrier patient, and bladder cancer in a BRCA Mexican founder mutation carrier.

Outcomes (10). Hollis and colleagues summarized the evidence regarding the implications of different germline BRCA PVs in ovarian cancer and concluded that aberrations at particular BRCA1 sites could confer differential sensitivity to platinum-based chemotherapy and PARPi (14). Furthermore, a study of patients with ovarian cancer showed that the presence of BRCA2 PVs in the ovarian cancer cluster region was associated with a better RFS than those in breast cancer cluster regions and not-related risk regions (25). Drost and colleagues investigated mice carrying the BRCA1185stop and BRCA15382stop alleles (which mimic the most common human BRCA1 founder mutations) and showed that the BRCA1185stop mice responded markedly worse to HRR deficiency–targeting therapies (13). Together, these studies suggest that not all BRCA PVs have the same impact on tumor phenotype, treatment response, or survival outcomes.

Reversion mutations are secondary alterations in a mutant allele that restore partial or complete protein functionality by reverting an initial frameshift mutation into an in-frame internal deletion (26). In BRCA-associated breast tumors, the appearance of acquired somatic reversion mutations has been described as a potent oncogenic event to resist unwanted DNA damage though the restoration of BRCA expression (27). Thus, the development of reversion mutations in BRCA PV carriers with breast cancer could have an adverse prognostic impact by limiting the effectiveness of DNA-damaging treatment strategies such as platinum-based chemotherapy regimens. The clinical significance of reversion mutations was illustrated by Lin and colleagues in a study where the absence of BRCA reversion mutations in circulating cell-free DNA of patients with ovarian cancer was associated with a significantly longer rucaparib progression-free survival (16). Recently, it was reported in a small study that patients with ovarian cancer harboring the Mexican BRCA1 founder CNVs had a better RFS than those with other types of BRCA1 PVs (25). However, the prognostic role of CNV such as the Mexican BRCA1 founder mutation on BC had not been previously reported. We postulate that the significantly enhanced OS in the BRCA1 ex9–12del PV carrier group found in our cohort occurs secondary to the inability of reversion mutations to restore the vast deletion associated with this CNV. We believe that patients with TNBC who carry this PV could tend toward sustained responses to DNA-damaging therapies (such as chemotherapy regimens based on the use of platinum or anthracyclines plus alkylating agents; refs. 28, 29), and this may also relate to trend in survival when comparing CNV carriers to noncarriers. However, the precise mechanisms underlying the observed differences among outcomes among patients with BRCA PV carriers were not examined in the current study and may represent a zone of future investigation.

TABLE 3 Survival outcomes at 5 and 10 years according to BRCA status.

| BRCA PVs carriers | All BRCA PV carriers | BRCA CNV carriers | Other BRCA PVs carriers | Noncarriers of BRCA PVs |
|-------------------|----------------------|-------------------|------------------------|-------------------------|
| Outcome           | OS At 5 years        | 90.7% (77.1–96.4%)| 100% (90.5–100%)       | 84.6% (64.0–93.9%)     |
|                   | At 10 years          | 87.3% (71.8–94.6%)| 100% (73.5–100%)       | 78.6% (55.0–90.7%)    |
|                   | RFS At 5 years       | 86.1% (71.6–93.5%)| 94.1% (65.0–99.2%)     | 80.8% (59.8–91.5%)    |
|                   | At 10 years          | 83.6% (68.6–91.8%)| 87.8% (59.5–96.8%)     | 80.8% (59.8–91.5%)    |

NOTE: Data are shown as Kaplan–Meier estimate (95% CI).
Mexican BRCA1 founder mutation carriers and other BRCA PVs are presently unknown and warrant further study.

Our study has several limitations. Full sequencing for BRCA PVs was not undertaken and was limited to those included in the HISPANEL assay (sensitivity 77%; ref. 24). Therefore, some of the patients classified as noncarriers of BRCA mutations could carry PVs that were not detected using our methodology. However, we believe any bias introduced by misattributed cases would not affect the survival analysis of CNV carriers versus other PV carriers. In addition, the HISPANEL assay does not identify PVs in other breast cancer susceptibility genes, which could have been present in some of our patients. Few panel genes (e.g., PALB2) have evidence for impact on survival or evidence for benefit from DNA-damaging therapies. Again, misattribution would not affect the survival advantage observed in CNV carriers compared with other PV carriers. The cohort selected for this analysis is limited by the small sample size and could be subject to survivorship bias as a median of 37.5 months had elapsed from breast cancer diagnosis to study inclusion. Therefore, patients with poor survival could have been disproportionately excluded, and this could be responsible in part for the relatively favorable outcomes for the entire study population. However, as all groups were subject to the same bias, we believe the study’s subsequent prospective outcomes are still valid. Moreover, the follow-up after inclusion was long, and OS and RFS calculated from time of study accrual demonstrated the same tendencies toward improved survival in the carriers of BRCA CNV group. Notably, all deaths registered in BRCA PV carriers were attributable to breast cancer. Hence, breast cancer-specific survival was essentially equivalent to OS in BRCA carriers, and the differences we observed were not due to mortality from a second primary malignancy. Nonetheless, it is possible that the benefit observed in survival outcomes in CNV carriers was influenced by a lower prevalence of lymph node invasion and stage III disease. The only large CNV observed in our cohort was the Mexican BRCA1 founder mutation, which limits the extrapolation of the prognostic impact of CNVs observed in this study to other BRCA CNVs. However, we suggest that any CNV with a comparably large deletion would limit the potential for reversion mutations and result in improved survival. Finally, due to the retrospective nature of this study, no adjustment for multiple hypothesis testing was performed. Hence, an inflation of type I error due to multiple testing cannot be excluded. Combined with the issues listed above, this underscores the need to interpret the results of this study as hypothesis-generating and independent validation is required. Despite these limitations, we believe that this analysis provides evidence supporting a survival impact from specific BRCA genotypes, with a plausible mechanistic underpinning.

In conclusion, this study demonstrates that the type of germline BRCA PV can influence survival after treatment for BC. We demonstrated that patients with TNBC with the BRCA1 ex9–12del had statistically better OS than carriers of other BRCA PVs. This could have important prognostic and clinical
implications. Future studies are needed to confirm these findings and test if the observed survival differences are due to different susceptibility to reversion mutations or other factors.

Authors’ Disclosures

C. Villarreal-Garza reports grants and personal fees from AstraZeneca; grants, personal fees, and non-financial support from Roche; personal fees from Myriad Genetics, Novartis, Eli Lilly; personal fees and non-financial support from Pfizer, and personal fees and non-financial support from MSD Oncology outside the submitted work. P. Frankel reports grants from City of Hope “NIH Grant Support” during the conduct of the study; J.N. Weitzel reports grants from NCI during the conduct of the study; personal fees from AstraZeneca “Speakers bureau” outside the submitted work. No disclosures were reported by the other authors.

Authors’ Contributions

C. Villarreal-Garza: Conceptualization, resources, data curation, formal analysis, investigation, writing-original draft, project administration, writing-review and editing. A.S. Ferrigno: Data curation, formal analysis, investigation, writing-original draft, writing-review and editing. N.H. Ruel: Data curation, formal analysis, investigation, writing-review and editing.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71: 209-49.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68: 394-424.
3. Yoshida R. Hereditary breast and ovarian cancer (HBOC): review of its molecular characteristics, screening, treatment, and prognosis. Breast Cancer 2021;28: 1167-80.
4. Spurdle AB, Couch FJ, Parsons MT, McGuffog L, Barrowdale D, Bolla MK, et al. Refined histopathological predictors of BRCA1 and BRCA2 mutation status: a large-scale analysis of breast cancer characteristics from the BCAC, CIMBA, and ENIGMA consortia. Breast Cancer Res 2014;16: 3419.
5. Zhu Y, Wu J, Zhang C, Sun S, Zhang J, Liu W, et al. BRCA mutations and survival in breast cancer: an updated systematic review and meta-analysis. Oncotarget 2016;7: 70113-27.
6. Zhong Q, Peng HL, Zhao X, Zhang L, Hwang WT. Effects of BRCA1- and BRCA2-related mutations on ovarian and breast cancer survival: a meta-analysis. Clin Cancer Res 2015;21: 211-20.
7. Van Den Broek AJ, Schmidt MK, Van ’t Veer LJ, Tollenaar R, Van Leeuwen FE. Worse breast cancer prognosis of BRCA1/BRCA2 mutation carriers: what’s the evidence? A systematic review with meta-analysis. PLoS One 2015;10: e0120189.
8. Vocka M, Zimovjanova M, Bielcikova Z, Tesarova P, Petruzelka L, Mateju M, et al. Estrogen receptor status opposite modifies breast cancer prognosis in brcal/brc2 mutation carriers versus non-carriers. Cancers (Basel) 2019;11: 738.
9. Templeton AJ, Gonzalez LD, Vera-Badillo FE, Tibau A, Goldstein R, Séruga B, et al. Interaction between hormonal receptor status, age and survival in patients with BRCA1/2 germline mutations: a systematic review and meta-regression. PLoS One 2016;11:e0154789.
10. Xie Y, Gou Q, Wang Q, Zhong X, Zheng H. The role of BRCA status on prognosis in patients with triple-negative breast cancer. Oncotarget 2017;8: 8751-62.
11. Yadav S, Ladkany R, Yadav D, Alhabib O, Khaddam S, Isaac D, et al. Impact of BRCA mutation status on survival of women with triple-negative breast cancer. Clin Breast Cancer 2018;18: e1229-35.
12. Copson ER, Maichman TC, Tapper WJ, Cutress RI, Greville-Heygate S, Altman DG, et al. Germline BRCA1 mutation outcome in young-onset breast cancer (POSH): a prospective cohort study. Lancet Oncol 2018;19: 169-80.
13. Drost R, Dhillon KK, Van Der Gulden H, Van Der Heijden I, Brandsma I, Cruz C, et al. BRCA1/185delAG tumors may acquire therapy resistance through expression of RING-less BRCA1. J Clin Invest 2016;126: 2903-18.
14. Hollis RL, Churchman M, Gourley C. Distinct implications of different BRCA mutations: Efficacy of cytotoxic chemotherapy, PARP inhibition and clinical outcome in ovarian cancer. Onco Targets Ther 2017;10: 2539-51.
15. Forbes C, Fayter D, Keck SK, Quek RGW. A systematic review of international guidelines and recommendations for the genetic screening, diagnosis, genetic counseling, and treatment of BRCA-mutated breast cancer. Cancer Manag Res 2019;11: 2321-37.
16. Lin KK, Harrell MI, Oza AM, Oaknin A, Ray-Coquard I, Tinker AV, et al. BRCA reversion mutations in circulating tumor DNA predict primary and acquired resistance to the PARP inhibitor rucaparib in high-grade ovarian carcinoma. Cancer Discov 2019;9: 210-9.
17. Gogola E, Rottertingen S, Jonkers J. Resistance to PARP inhibitors: lessons from preclinical models of BRCA-associated cancer. Annu Rev Cancer Biol 2019;3: 235-54.
18. James PA, Sawyer S, Boyle S, Young MA, Kovalenko S, Doherty R, et al. Large genomic rearrangements in the familial breast and ovarian cancer gene BRCA1 are associated with an increased frequency of high risk features. Fam Cancer 2015;14: 287-95.
19. Montagna M, Palma MD, Menin C, Agata S, De Nicolao A, Chioco-Bianchi L, et al. Genomic rearrangements account for more than one-third of the BRCA1 mutations in northern Italian breast/ovarian cancer families. Hum Mol Genet 2003;12: 1055-61.
20. Weitzel JN, Lagos VI, Herzog JS, Judkins T, Hendrickson B, Ho JS, et al. Evidence for common ancestral origin of a recurring BRCA1 genomic rearrangement.
identified in high-risk hispanic families. Cancer Epidemiol Biomarkers Prev 2007;16: 1615-20.

21. Villarreal-Garza C, Weitzel JN, Liacuachaqui M, Sifuentes E, Magailanes-Hoyos MC, Gallardo L, et al. The prevalence of BRCA1 and BRCA2 mutations among young Mexican women with triple-negative breast cancer. Breast Cancer Res Treat 2015;150: 389-94.

22. Lara-Medina F, Pérez-Sánchez V, Saavedra-Pérez D, Blake-Cerda M, Arce C, Motola-Kuba D, et al. Triple-negative breast cancer in Hispanic patients: high prevalence, poor prognosis, and association with menopausal status, body mass index, and parity. Cancer 2011;117: 3658-69.

23. Reynoso-Noverón N, Villarreal-Garza C, Soto-Perez-de-Celis E, Arce-Salinas C, Matus-Santos J, Ramirez-Ugalde MT, et al. Clinical and Epidemiological Profile of Breast Cancer in Mexico: Results of the Seguro Popular. J Glob Oncol 2017;3: 757-64.

24. Villarreal-Garza C, Alvarez-Gómez RM, Pérez-Plasencia C, Herrera LA, Herzog J, Castillo D, et al. Significant clinical impact of recurrent BRCA1 and BRCA2 mutations in Mexico. Cancer 2015;121: 372-8.

25. Gallardo-Rincón D, Álvarez-Gómez RM, Montes-Servín E, Toledo-Leyva A, Montes-Servín E, Michel-Tello D, et al. Clinical Evaluation of BRCA1/2 Mutation in Mexican Ovarian Cancer Patients. Transl Oncol 2020;13: 212-20.

26. Ganesan S. Tumor Suppressor Tolerance: Reversion Mutations in BRCA1 and BRCA2 and Resistance to PARP Inhibitors and Platinum. JCO Precis Oncol 2018;2: 1-4.

27. Hatano Y, Tamada M, Matsuo M, Hara A. Molecular Trajectory of BRCA1 and BRCA2 Mutations. Front Oncol 2020;10: 361.

28. Tung N, Arun B, Hacker M, Hofstatter E, Toppmeyer D, Isakoff S, et al. TBCRC 031: Randomized Phase II Study of Neoadjuvant Cisplatin Versus Doxorubicin-Cyclophosphamide in Germline BRCA Carriers With HER2-Negative Breast Cancer (the INFORM trial). J Clin Oncol 2020;38: 1539-48.

29. Pohl-Rescigno E, Hauke J, Loibl S, Möbus V, Denkert C, Fasching P, et al. Association of Germline Variant Status With Therapy Response in High-risk Early-Stage Breast Cancer: A Secondary Analysis of the GeparOcto Randomized Clinical Trial. JAMA Oncol 2020;6: 744-8.