Prevalence of tumor genomic alternations in homologous recombination genes among Taiwanese breast cancers

Chi-Cheng Huang

Taipei Veterans General Hospital

Chun-Yu Liu

Taipei Veterans General Hospital

Yi-Fang Tsai

Taipei Veterans General Hospital

Pei-Ju Lien

Taipei Veterans General Hospital

Yen-Shu Lin

Taipei Veterans General Hospital

Ta-Chung Chao

Taipei Veterans General Hospital

Chin-Jung Feng

Taipei Veterans General Hospital

Yen-Jen Chen

Taipei Veterans General Hospital

Jiun-I Lai

Taipei Veterans General Hospital

Nam Nhut Phan

National Taiwan University

Chih-Yi Hsu

Taipei Veterans General Hospital

Jen-Hwey Chiu

Taipei Veterans General Hospital

Ling-Ming Tseng (✉ lmstseng@vghtpe.gov.tw)

Comprehensive Breast Health Center and Department of Surgery, Taipei Veterans General Hospital, Taiwan, ROC

<https://orcid.org/0000-0002-4648-6646>

Research Article

Keywords: Taiwanese breast cancer, BRCA1/2 mutation, PARP inhibitor, synthetic lethality, homologous recombination repair

Posted Date: April 14th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-366269/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Purpose Deleterious *BRCA1 / 2* mutations are among the most highly pathogenic variants in hereditary breast and ovarian cancer syndrome. PARP (poly ADP ribose polymerase) inhibitors selectively cause failure of single-strand but does not affect double-strand DNA break repair, and ant-tumor activity is observed in *BRCA* mutant breast cancers. Recently genes implicated into the homologous recombination repair (HRR) pathways are investigated extensively, as defective HRR genes may indicate potential clinical benefits from PARP inhibitors beyond *BRCA1 / 2* mutations. Materials and Methods We evaluated the prevalence of *BRCA1 / 2* mutations as well as alternations in HRR genes for Taiwanese breast cancers with targeted sequencing. Consecutive 648 breast cancer samples were assayed, and HRR genes by Heeke et al. and those interrogated in Talazoparib Beyond *BRCA* (TBB) trial were evaluated for prevalence from breast cancer tissues. Results Among 648 breast cancer samples, there were 18 truncating and 2 missense mutations in *BRCA1* and 48 truncating and 2 missense mutations in *BRCA2*, impacting 3% and 5% of study population (collectively altered in 6%) with co-occurrence of *BRCA1 / 2* in 7 breast cancers. On the other hand, HRR genes defined by Heeke et al. were altered in 122 (19%) breast cancers while TBB interrogated genes (excluding *BRCA1 / 2*) were mutated in 107 (17%) patients. Beyond *BRCA1/2*, the most prevalent HRR mutant genes came from *ARID1A* (7%), *PALB2* (7%) and *PTEN* (6%). Collectively, 164 (25%) of the 648 Taiwanese breast cancer samples were impacted by at least one HRR gene. Conclusion The prevalence of high-penetrant *BRCA1 / 2* mutations was far below one tenth of assayed samples while the prevalence of tumor DNA mutations in HRR pathways was much higher and approached one fifth among Taiwanese breast cancers. Further studies to evaluate the efficacy of PARP inhibitors in patients with defective HRR gene(s) are warranted to broaden the targeted population of synthetic lethality.

Introduction

Breast cancer has overtaken lung cancer as the most common cancer, accounting for nearly 12% of new cases each year worldwide, according to the World Health Organization [1]. Breast cancer is also one of the leading causes of cancer-related death for women in Taiwan, and the annual incidence is continuously increasing throughout the past two decades [2–3]. Outcomes of breast cancer treatment have improved enormously with long-term survival achieved due to awareness, wide spread of screening and efficient therapeutic options. With advocacy of personalized medicine, the combination of clinical and genetic information is useful for decision making and precise selection of targeted agents [4].

Among all breast cancer susceptible genes, *BRCA1/2* deletions are among the most highly pathogenic genetic alternations in hereditary breast and ovarian cancer (HBOC) syndrome [5]. Pathogenic/likely pathogenic *BRCA* mutations not only trigger genetic counseling for the infected proband and associated family members, germline mutations of *BRCA* are predictive biomarkers for synthetic lethargy [6–8]. PARP (poly ADP ribose polymerase) inhibitors selectively cause failure of DNA single-strand break (SSB) repair but does not affect double-strand break (DSB) repair, for which intact *BRCA1* and *BRCA2* are indispensable component enzymes within cascading pathway [9]. Under the circumstance of defective *BRCA1/2* deleterious mutations, anti-tumor activity is observed with the treatment of PARP inhibitors [10].

Our previous study has evaluated mutational profiles of *BRCA1/2* among Taiwanese breast cancers, and the preliminary results of 100 consecutive patients assayed with targeted sequencing of tumor-only samples showed 74 single nucleotide variants (SNVs), 2 copy number variations (CNVs), and 15 insertions/deletions (indels) with a

prevalence rate of 4% (4 breast cancers). Regarding the distribution of filtered SNVs, 38 were with *BRCA2* original and 36 with *BRCA1* gene, while 40 out of them were reported as being benign or likely benign, 33 as variant of uncertain significance (VUS) or never referred, and 1 pathogenic. The only pathogenic SNV, *BRCA1* (c.3607C > T), resulting in p.Arg1203Ter amino acid change from one triple negative breast cancer (TNBC). Additional four pathogenic variants were identified from four indels among three breast cancers. The doublet *BRCA2* pathogenic indels (c.1612_1613insA and c.1792_1793insA; amino acid changes: p.Ser538fs and p.Thr598fs) came from one premenopausal TNBC patient with a positive family history. The remaining pathogenic *BRCA2* (c.5994delA; p.Val1999fs) and *BRCA1* deletion (c.2110_2111delAA; p.Asn704fs) were revealed from two luminal/human epidermal growth factor receptor II (HER2)-negative breast cancers [11].

Taking our series of non-selective 100 consecutive Taiwanese cases together with others, the prevalence of somatic/germline mutations of *BRCA1/2* is extremely low and never exceed one-tenth, even among TNBC, further limiting the potential beneficiaries of synthetic lethargy [12–13]. Recently genes implicated in the homologous recombination repair (HRR) pathways are investigated extensively, as defective HRR genes other than *BRCA1* and *BRCA2* may indicate potential applications from PARP inhibitors to alleviate the prerequisite of defective BRCA genes [14]. In current study we evaluated the prevalence of mutations within *BRCA1/2* and other HRR genes among Taiwanese breast cancers.

Materials And Methods

The aim of the study was to evaluate the prevalence of *BRCA1/2* mutations as well as mutations of HRR genes for Taiwanese breast cancers with targeted sequencing of tumor-only samples.

Study population

The full protocol of the VGH-TAYLOR: Comprehensive precision medicine research on the heterogeneity of Taiwanese breast cancer patients, and results of initial 380 targeted sequencing have been described elsewhere [15]. The study comprised a broad clinical spectrum of breast cancers, including Group 1: planned to receive first-line surgery and followed by adjuvant therapy, or early relapse within three years. Group 2: planned to receive first-line neoadjuvant therapy and followed by surgery, and Group 3: de novo stage IV, or stage IV with recurrence beyond three years. Three years of enrollment and four years follow-up after enrollment were planned.

For clinical parameters, estrogen receptor (ER) and progesterone receptor (PR) positive was defined as $\geq 1\%$ of tumor cells exhibiting nuclear staining by immunohistochemistry (IHC) assay. Patients with HER2 testing scored as IHC 3+ (positive) or 2+ (equivocal) and with fluorescence in situ hybridization (FISH) confirmed amplification were regarded as being HER2 positive. For molecular subtyping, luminal A subtype was defined as ER positive, HER2 negative, with PR $\geq 20\%$, Ki-67 $\leq 20\%$, and nuclear differentiation as grade I or II. Luminal B1 breast cancers were ER positive, HER2 negative and at least one held true for PR < 20%, Ki-67 > 20%, or being nuclear poorly differentiated as grade 3. Luminal B2 breast cancers were those with both ER and HER2 positive phenotypically.

All patients received treatment following the contemporary practice guidelines of Comprehensive Breast Health Center at Taipei Veterans General Hospital, which was based on the NCCN and St. Gallen guidelines [16-18].

HR-related genes list

Candidates of HRR genes were based on those reported by Heeke et al. (*ARID1A*, *ATM*, *ATRX*, *BAP1*, *BARD1*, *BLM*, *BRCA1/2*, *BRIP1*, *CHEK1/2*, *FANCA/C/D2/E/F/G/L*, *MRE11A*, *NBN*, *PALB2*, *RAD50*, *RAD51*, *RAD51B*, *WRN*) and those interrogated in the Talazoparib Beyond BRCA (TBB) trial (*PALB2*, *CHEK2*, *ATM*, *NBN*, *BARD1*, *BRIP1*, *RAD50*, *RAD51C*, *RAD51D*, *MRE11*, *ATR*, *PTEN*, *FANCA/C/D2/E/F/G/L*) [19-20].

Targeted sequencing

Details of targeted sequencing has been described elsewhere and the Ion Torrent Oncomin Comprehensive (OCP) Assay v3 (Thermo Fisher Scientific, Waltham, MA) was adopted for next-generation sequencing (NGS), which enabled detections of 161 cancer-related genes and identified SNVs, CNVs, gene fusions, and indels [15]. Formalin-fixed paraffin-embedded (FFPE) specimens were assayed; sequencing data were analyzed, aligned and annotated through the Torrent Suit (Thermo Fisher Scientific) and Ion Reporter (Thermo Fisher Scientific) software with the default OncoPrint BRCA (5.12) filter applied.

Variant caller and annotation

Mutational consequences of called variants were ascertained with ClinVAR [21]. To further correct spurious findings due to trans-ethnic discrepancy, the on-line VariED tool was consulted to filter out Taiwan Biobank polymorphisms [22].

Results

Enrolled Taiwanese breast cancers

In current study, we presented the updated results of 648 TMO assays from 621 breast cancer patients (27 assayed twice) from the VGH-TAYLOR study. The distributions of clinical scenarios were: Group 1A (surgery first, n=387), Group 1B (recurrence within 3 years, n=25), Group 2 (neoadjuvant therapy first, n=93), Group 3-1 (de novo stage IV, n=36), and Group 3-2 (recurrence beyond 3 years, n=36). In addition, there were 71 subjects from

retrospective biobank cohort. Twenty-eight patients were assayed twice, including 19 in Group 2 (diagnostic/post-neoadjuvant pair) and 8 in Group 1B. Fig 1 displays the distributions of IHC results and molecular subtypes.

Mutational maps of *BRCA1/2*

Among 648 breast samples, there were 18 truncating and 2 missense mutations in *BRCA1* and 48 truncating and 1 missense mutations in *BRCA2*, impacting 3% (n=18) and 5% (n=29) of study cohort respectively (collectively altered in 6%, or 40 patients, Fig 2). Recurrent (observed at least twice) pathogenic/likely pathogenic mutations included W321*, K830fs, R1203*, Q1577* (*BRCA1*) and R3052Q, Q2829*, R2520*, S2186fs, K1472fs, S1722fs (*BRCA2*), with co-occurrence of *BRCA1/2* in 7 breast cancers (log2 odds ratio > 3, both p- and q-value < 0.001, Fig 3). Regarding IHC phenotypes, there were 7 hormone receptor (HR)+/HER2- (39%), 2 HR+/HER2+ (11%), 2 HR-/HER2+ (11%), and 7 HR-/HER2- (39%) *BRCA1* mutant breast cancers while for *BRCA2* mutated cases, 19 (66%) were HR+/HER2-, 4 (14%) were HR+/HER2+, 3 (10%) were HR-/HER2+, and 3 (10%) were HR-/HER2-. It deserves notice that one HR+/HER2- patient harbored dual *BRCA1* mutations (W1836* and A1729V), one HR+/HER2+ patient harbored triple *BRCA2* truncating mutations (Q126*, Q1124*, Q3295*), four HR+/HER2- subjects with multiple *BRCA2* mutations (range: 2-8), one HR+/HER2+ with dual *BRCA2* mutations (Q649* and Q2829*), and another one HR-/HER2- with triple *BRCA2* frameshift mutations (S538fs, T598fs, S973fs).

Among the 7 cases who were mutated in both *BRCA1/2*, three retrospective cohort patients including one HR+/HER2- with *BRCA1* (Q1577*) and *BRCA2* (R2520* and R3052Q), one HR-/HER2+ with *BRCA1* (Q1867*) and *BRCA2* (Q649* and Q2829*), and one HR+/HER2- with *BRCA1* (R1720Q) and *BRCA2* (W2970*) were reported. One Group 1B HR+/HER2+ with *BRCA1* (Q855*) and *BRCA2* (Q126*, Q1124*, Q3295*), one Group 3-1 HR-/HER2- with *BRCA1* (S1180fs) and *BRCA2* (S538fs, T598fs, S973fs), one Group 3-2 HR+/HER2- with *BRCA1* (W321*) and *BRCA2* (Q66*, Q2100*, E2220fs, Q2491*, R2494*, Q2506*, Q2823*, W2990*), and another one Group 3-2 HR+/HER2- with *BRCA1* (W1836*, A1729V) and *BRCA2* (Q1138*, Q2024*, W2626*) were also identified. Supplementary Fig 1 shows the chord diagram.

HRR genes

HRR genes defined by Heeke et al. were altered in 122 (19%) breast cancer samples while TBB interrogated genes (excluding *BRCA1/2*) were mutated in 107 (17%, Fig 4). Collectively, 164 (25%) of the 648 Taiwanese breast cancer samples were impacted by at least one HRR gene (Table 1 shows clinical presentations). The number of mutant HRR genes per sample ranged from 1 to 16 (median: 1, SD: 2.3) while 29 cases harbored more than one HRR mutated genes. Beyond *BRCA1/2*, the most prevalent HRR mutations came from *ARID1A* (7%), *PTEN* (7%), and *PALB2* (6%), with the remaining genes impacting fewer samples than either of *BRCA1/2* genes (Supplementary Fig 2). Clinically, there were 30 (75%) HR+/HER2-, 4 HR+/HER2+ (10%), 3 (8%) HR-/HER2+ and 3 (8%) HR-/HER2- *ARID1A* mutant breast cancers while four HR+/HER2- (G869fs/G869S, P225fs/P533fs,

Q575*/Q1364*, Q743fs/E1718fs/S2256*), one HR+/HER2+ (Q199*, Q775*, Q1366*), one HR-/HER2+ (Q581*, Q670*, Q844*) and one HR-/HER2- (Q1363*, W1686*) breast cancers showed more than one *ARID1A* mutation within the same patient. The IHC distributions (HR+/HER2-:HR+/HER2+:HR-/HER2+:HR-/HER2-) for *PTEN* and *PALB2* were 27(68%):1(3%):2(5%):10(25%) and 28(72%):2(5%):3(8%):6(15%) while multiple mutations per subject were noted in 9 HR+/HER2-, one HR+/HER2+ and another HR-/HER2+ *PTEN* mutant breast cancers as well as 6 HR+/HER2- and one HR-/HER2- *PALB2* mutant cases. Trend of mutual exclusivity was observed between *BAP1* and *PALB2* (log2 odds ratio < -3, p-value: 0.717, q-value: 0.722) as well as *BAP1* and *RAD51C* (log2 odds ratio < -3, p-value: 0.928, q-value: 0.928) while most HRR genes showed some degree of co-occurrence (Fig 5 shows the chord diagram). Mutation map of HRR genes other than *BRCA1/2* is detailed in Supplementary Fig 3.

Discussion

Breast cancer is one of the leading causes of cancer-related death in women, and the annual incidence of breast cancer has increased continuously throughout the past two decades in Taiwan. There is a demand to identify therapeutics based on genetic profiling from breast cancer samples to improve treatment response and suppress recurrence rate. Our previous study has determined the molecular alternations underpinning breast oncogenesis and identified potential biomarkers from a large cohort of Taiwanese breast cancers using tumor-only targeted sequencing [15]. In current study, we focused on the prevalence of HRR genes to shed light on the potential application of PARP inhibitors and subsequent synthetic lethargy.

The prevalence of *BRCA1/2* mutations (ESMO Scale for Clinical Actionability of molecular Targets (ESCAT) Tier 1A for germline and Tier IIIA for somatic mutation) in an unselected breast cancer cohort was collectively 6% in Taiwan [23]. *BRCA1/2* mutation status does not confer adverse prognosis other than contralateral breast cancer occurrence, and no disease-specific or overall survival difference is observed between *BRCA1/2* mutant and wild-type breast cancers [24–25]. Synthetic lethality is evidenced when occurrence in either of the two genetic events individually has no effect but combining both events leads to cell death. PARP inhibitor selectively causes failure of SSB but does not affect DSB repair pathway, and durable ant-tumor response was observed in patients with *BRCA* mutant breast cancers [26]. Both triple negative (HR-/HER2-) and HR+/HER2- breast cancers may benefit from the defective DSB repair pathway inherited from a deleterious *BRCA* gene mutation, and the recently approved PARP inhibitor could be a reasonable therapeutic choice in metastatic or recurrent scenarios after adjuvant chemotherapy. FDA-approved PARP inhibitors such as olaparib and talazoparib were responsive for advanced/metastatic breast cancers who had undergone previous chemotherapy if germline mutation was also confirmed [27–28]. Mechanisms of PARP inhibitor comes from targeting DNA damage response, destabilizing replication forks through PARP-DNA entrapment and inducing cell death through replication stress-induced mitotic catastrophe [6]. Breast cancers with *BRCA1/2* mutations are candidates for synthetic lethargy if their mutations are proved germline origin. Low mutation rate of *BRCA1/2* in sporadic/hereditary breast cancers, however, further limit the clinical applicability of PARP inhibitors.

To overcome the low prevalence of germline *BRCA1* and *BRCA2* mutations, numerous efforts have been made to evaluate the role of other DNA damage repair (DDR) genes beyond *BRCA1/2*, with the hope to extend the clinical applicability of PARP inhibitors [29]. Indeed, there are multiple players involved in the HRR pathways, such as sensors of DSB (*ATM* and *ATR*) which subsequently lead to activation signal mediators (*BRCA1*, *BRCA2* and *PALB2*), as well as nuclear protein *RAD51* for strand invasion and replication fork stabilization [30]. Dozens of genes have been reported as being interrogated within the HRR process, and a critical question raised is to

determine an operable and reproducible genes list as a predictive biomarker for targeted therapy. It's also of great clinical potentiality to treat *BRCA*-wild breast cancers that can benefit from PARP inhibitors based on HRR deficiency.

In current study, two definitions of HRR genes were adopted. Heeke et al. evaluated 56,426 solid tumors molecularly profiled with NGS or Sanger sequencing. HRR deficiency by lineage was reported, with an overall mutation frequency of 17.4% reported [19]. Overall, the most frequently mutated genes were *ARID1A* (7.2%), *BRCA2* (3%), *BRCA1* (2.8%), *ATM* (1.3%), *ATR* (1.3%), *CHEK2* (1.3%) and *BAP1* (1.1%). Breast cancer ranked the ninth over 21 cancer types in terms of HRR deficiency while all metastatic solid tumors should be profiled according to their conclusion. We also evaluated HRR genes purposed by the TBB study, which was a phase II trial evaluating talazoparib in advanced HER2-negative breast cancer or other solid tumors with a germline or somatic alteration in HRR genes excluding *BRCA1/2* [20]. Clinical benefits were observed for breast cancers with germline *PALB2*, *CHEK1*, *FANCA* and somatic *PTEN*, *ATR* mutations. In our tumor-only series, *BRCA1/2* mutations collectively impacted 6% of Taiwanese breast cancers, and the proportion with mutant HRR genes ranged between 17% and 19% based on different definition, and a total of 25% Taiwanese breast cancers harbored at least one alternation among HRR gene from targeted sequencing. Another proof of concept study comes from the TCBRC048 trial, which reported response rate of 82% among 11 germline *PALB2* mutant breast cancers and 50% for 16 somatic *BRCA1/2* mutant cases with olaparib [31].

It's interesting to see if there exists a dose-dependent effect of PARP inhibition based on dual *BRCA* alternations or doublet/triplet mutations of *BRCA1/2* as our study revealed co-occurrence of *BRCA1* and *BRCA2* in seven Taiwanese breast cancers as well as one doublet *BRCA1* and seven cases with multiple *BRCA2* mutations. Clinical phenotypes do matter here as PARP inhibitors are indicated only for HER2 negative advanced/metastatic breast cancers. Although up to one-fourth of Taiwanese breast cancers reported tumor mutations in HRR genes, this did not necessarily translate into clinical actionability under current evidence of targeted therapy. Nowadays only germline *BRCA1/2* mutations have therapeutic implications. Ongoing clinical trials are evaluating PARP inhibitors in other DDR pathway genes (both germline and somatic); the clinical significance of somatic *BRCA1/2* mutations in breast cancer remains inconclusive, and the ongoing NCT03344965 and VIOLETTE (NCT03330847) trials are designed to answer the question whether *BRCA1/2* somatic mutation could act as a predictive marker for PARP inhibitor response as olaparib in ovarian cancer [32–34].

In addition to PARP inhibitor monotherapy, the combination with immune checkpoint blockade such as TOPACIO (niraparib plus pembrolizumab in triple negative breast cancer regardless of *BRCA1/2* status), MEDIOLA (olaparib plus durvalumab in triple negative breast cancer with germline *BRCA1/2* mutation), DORA (olaparib plus durvalumab as maintenance therapy after platinum chemotherapy), NCT03801369 (olaparib plus durvalumab in *BRCA*-wild type triple negative breast cancer) and JAVELIN *BRCA/ATM* (avelumab plus talazoparib with either *BRCA* or *ATM* mutation) have been extensively investigated [35]. The combination of cisplatin chemotherapy with a less potent PARP inhibitor (veliparib) was evaluated in the SWOG1416 trial, and for the first time that an improvement in progression-free and overall survival with a PARP inhibitor in metastatic triple negative breast cancer without a germline *BRCA* mutation had been demonstrated [36]. It was the “*BRCA*-like” group which derived the most clinical benefit from the combination therapy. Four biomarkers, HRD genomic instability score with cutoff 42, somatic *BRCA1/2* mutation, *BRCA1* promoter methylation, and germline HRR gene mutations excluding *BRCA1/2*, constituted the *BRCA*-like phenotype [37].

There were some limitations of the study. First, we conducted tumor-only sequencing while reflex testing for germline mutations were not carried out once alternations in HRR genes were reported. The tumor-only study design abolished the chance to discover germline mutations from targeted sequencing [38]. Without matched germline sequencing, it was difficult to differentiate somatic from germline mutations for investigated genes. Second, although the commercialized OCP assay was used with wide coverage of tumor associated genes, there were still missing HRR genes not interrogated such as *BARD1*, *BLM*, *BRIP1*, *CHEK2*, *FANCC*, *FANCE*, *FANCF*, *FANCG*, *FANCL*, *MRE11A* and *WRN*. Most of these missing genes were of rare variants and rarely addressed by most targeted panels. Third, “genomic scar”, i.e. scores capturing large genomic aberrations such as loss of HRR using SNP arrays, myChoice HRD (Myriad Genetics) and Foundation Focus CDx BRCA LOH (Foundation Medicine) could not be mimicked by NGS experiments [39–40]. Only point mutations presented as SNVs, MNVs, Indels, as well as structural aberrations including CNVs and fusions were evaluated in HRR genes using tumor DNA sequencing.

Conclusion

Our study confirmed the feasibility of targeted sequencing with a commercial tumor-only panel while the prevalence of high-penetrant *BRCA1/2* mutations was collectively up to 6% of an unselected Taiwanese population, which narrowed the potentially targeted population of PARP inhibitors substantially. The prevalence of tumor DNA mutations in HRR pathway was much higher and approached one fifth among Taiwanese breast cancers. PARP inhibitor is a successful paradigm for synthetic lethargy and the frontline will be extended to early stage disease in near future [41]. Further studies to evaluate the efficacy of PARP inhibitors in patients with a defective HRR pathway are warranted to broaden the targeted population of synthetic lethality.

Acknowledgement

The study was exclusively sponsored by YongLin Healthcare Foundation under the clinical study protocol No. QCR18002. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. This manuscript contained materials presented during the Taipei International Breast Cancer Symposium 2020 Meeting during Nov 7 and 8, 2020.

References

1. Most Common Cancer Diagnosis Globally: Breast Surpasses Lung - Medscape - Dec 17 (2020) Robson M, Im SA, Senkus E, Xu B, Domchek SM, Masuda N, Delaloge S, Li W, Tung N, Armstrong A, Wu W, Goessl C, Runswick S, Conte P. Olaparib for Metastatic Breast Cancer in Patients with a Germline BRCA Mutation. *N Engl J Med* 2017;377:523–533. URL: <https://www.medscape.com/viewarticle/942808>. Accessed at Mar 17, 2021
2. Health Promotion Administration, Ministry of Health and Welfare, Taiwan. Cancer registry annual report, 2017, Taiwan. Taipei: Health Promotion Administration, Ministry of Health and Welfare, Taiwan, 2019
3. Department of Statistics, Ministry of Health and Welfare, Taiwan. Cause of death statistics, 2019, Taiwan, <https://www.mohw.gov.tw/cp-16-54482-1.html> (2019, accessed Sep-24, 2020)
4. Kuo CN, Liao YM, Kuo LN, Tsai HJ, Chang WC, Yen Y (2020 Dec) Cancers in Taiwan: Practical insight from epidemiology, treatments, biomarkers, and cost. *J Formos Med Assoc* 119(12):1731–1741

5. US Preventive Services Task Force, Owens DK, Davidson KW, Krist AH, Barry MJ, Cabana M, Caughey AB, Doubeni CA, Epling JW Jr, Kubik M, Landefeld CS, Mangione CM, Pbert L, Silverstein M, Simon MA, Tseng CW, Wong JB. Risk Assessment, Genetic Counseling, and Genetic Testing for BRCA-Related Cancer: US Preventive Services Task Force Recommendation Statement. *JAMA*. 2019 Aug 20;322(7):652–665
6. Slade D (2020 Mar) PARP and PARG inhibitors in cancer treatment. *Genes Dev* 1;34(5–6):360–394
7. Dziadkowiec KN, Gąsiorowska E, Nowak-Markwitz E, Jankowska A (2016 Dec) PARP inhibitors: review of mechanisms of action and BRCA1/2 mutation targeting. *Prz Menopauzalny* 15(4):215–219
8. Rose M, Burgess JT, O'Byrne K, Richard DJ, Bolderson E (2020 Sep) PARP Inhibitors: Clinical Relevance, Mechanisms of Action and Tumor Resistance. *Front Cell Dev Biol* 9:8:564601
9. Yi M, Dong B, Qin S, Chu Q, Wu K, Luo S (2019 Nov) Advances and perspectives of PARP inhibitors. *Exp Hematol Oncol* 11:8:29
10. Won KA, Spruck C (2020 Dec) Triple-negative breast cancer therapy: Current and future perspectives (Review). *Int J Oncol* 57(6):1245–1261
11. Huang CC, Tsai YF, Liu CY, Lien PJ, Wang YL, Lin YS, Chao TC, Feng CJ, Chiu JH, Chau GY, Tseng LM (2020) Mutational Profiles of BRCA 1/2 among Taiwanese Breast Cancers: Preliminary Results of 100 Consecutive Targeted Sequencing Samples. annual meeting of Taiwan Surgical Association
12. Zhong X, Dong Z, Dong H, Li J, Peng Z, Deng L, Zhu X, Sun Y, Lu X, Shen F, Su X, Zhang L, Gu Y, Zheng H. Prevalence and Prognostic Role of BRCA1/2 Variants in Unselected Chinese Breast Cancer Patients. *PLoS One*. 2016 Jun 3;11(6):e0156789
13. Armstrong N, Ryder S, Forbes C, Ross J, Quek RG (2019 Jul) A systematic review of the international prevalence of BRCA mutation in breast cancer. *Clin Epidemiol* 11:11:543–561
14. Wengner AM, Scholz A, Haendler B. Targeting DNA Damage Response in Prostate and Breast Cancer. *Int J Mol Sci*. 2020 Nov 4;21(21):8273
15. Huang CC, Tsai YF, Liu CY, Chao TC, Lien PJ, Lin YS, Feng CJ, Chiu JH, Hsu CY, Tseng LM (2021) Comprehensive molecular profiling of Taiwanese breast cancers revealed potential therapeutic targets: prevalence of actionable mutations among 380 targeted sequencing analyses. *BMC Cancer*. Feb 25;21(1):199
16. Burstein HJ, Curigliano G, Loibl S, Dubsy P, Gnant M, Poortmans P, Colleoni M, Denkert C, Piccart-Gebhart M, Regan M, Senn HJ, Winer EP, Thürlimann B; Members of the St. Gallen International Consensus Panel on the Primary Therapy of Early Breast Cancer 2019. Estimating the benefits of therapy for early-stage breast cancer: the St. Gallen International Consensus Guidelines for the primary therapy of early breast cancer 2019. *Ann Oncol* (2019) Oct 1;30(10):1541–1557
17. Curigliano G, Burstein HJ, Winer EP, Gnant M, Dubsy P, Loibl S, Colleoni M, Regan MM, Piccart-Gebhart M, Senn HJ, Thürlimann B; St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2017, André F, Baselga J, Bergh J, Bonnefoi H, Brucker SY, Cardoso F, Carey L, Ciruelos E, Cuzick J, Denkert C, Di Leo A, Ejlersen B, Francis P, Galimberti V, Garber J, Gulluoglu B, Goodwin P, Harbeck N, Hayes DF, Huang CS, Huober J, Hussein K, Jassem J, Jiang Z, Karlsson P, Morrow M, Orecchia R, Osborne KC, Pagani O, Partridge AH, Pritchard K, Ro J, Rutgers EJT, Sedlmayer F, Semiglazov V, Shao Z, Smith I, Toi M, Tutt A, Viale G, Watanabe T, Whelan TJ, Xu B. De-escalating and escalating treatments for early-stage breast cancer: the St. Gallen International Expert Consensus Conference on the Primary Therapy of Early Breast Cancer 2017. *Ann Oncol*. 2017 Aug 1;28(8):1700–1712

18. Telli ML, Gradishar WJ, Ward JH. NCCN Guidelines Updates: Breast Cancer. *J Natl Compr Canc Netw*. 2019 May 1;17(5.5):552–555
19. Heeke AL, Pishvaian MJ, Lynce F, Xiu J, Brody JR, Chen WJ, Baker TM, Marshall JL, Isaacs C. Prevalence of Homologous Recombination-Related Gene Mutations Across Multiple Cancer Types. *JCO Precis Oncol*. 2018;2018:PO.17.00286.
20. Gruber JJ, Afghahi A, Hatton A, Scott D, McMillan A, Ford JM, Telli ML. Talazoparib beyond BRCA: A phase II trial of talazoparib monotherapy in BRCA1 and BRCA2 wild-type patients with advanced HER2-negative breast cancer or other solid tumors with a mutation in homologous recombination (HR) pathway genes. *Journal of Clinical Oncology* 2019 37:15_suppl, 3006–3006
21. Landrum MJ, Chitipiralla S, Brown GR, Chen C, Gu B, Hart J, Hoffman D, Jang W, Kaur K, Liu C, Lyoshin V, Maddipatla Z, Maiti R, Mitchell J, O'Leary N, Riley GR, Shi W, Zhou G, Schneider V, Maglott D, Holmes JB, Kattman BL (2020) ClinVar: improvements to accessing data. *Nucleic Acids Res*. Jan 8;48(D1):D835-D844
22. Lee CY, Chattopadhyay A, Chiang LM, Juang JJ, Lai LC, Tsai MH, Lu TP, Chuang EY (2019) VariED: the first integrated database of gene annotation and expression profiles for variants related to human diseases. *Database (Oxford)*. Jan 1;2019:baz075
23. Mateo J, Chakravarty D, Dienstmann R, Jezdic S, Gonzalez-Perez A, Lopez-Bigas N, Ng CKY, Bedard PL, Tortora G, Douillard JY, Van Allen EM, Schultz N, Swanton C, André F, Puzsai L (2018) A framework to rank genomic alterations as targets for cancer precision medicine: the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT). *Ann Oncol*. Sep 1;29(9):1895–1902
24. De Talhouet S, Peron J, Vuilleumier A, Friedlaender A, Viassolo V, Ayme A, Bodmer A, Treilleux I, Lang N, Tille JC, Chappuis PO, Buisson A, Giraud S, Lasset C, Bonadona V, Trédan O, Labidi-Galy SI. Clinical outcome of breast cancer in carriers of BRCA1 and BRCA2 mutations according to molecular subtypes. *Sci Rep*. 2020 Apr 27;10(1):7073
25. Rennert G, Bisland-Naggan S, Barnett-Griness O, Bar-Joseph N, Zhang S, Rennert HS, Narod SA (2007) Clinical outcomes of breast cancer in carriers of BRCA1 and BRCA2 mutations. *N Engl J Med*. Jul 12;357(2):115 – 23
26. Lord CJ, Ashworth A. PARP inhibitors: Synthetic lethality in the clinic. *Science*. 2017 Mar 17;355(6330):1152–1158
27. Robson M, Im SA, Senkus E, Xu B, Domchek SM, Masuda N, Delaloge S, Li W, Tung N, Armstrong A, Wu W, Goessl C, Runswick S, Conte P (2017 Aug) Olaparib for Metastatic Breast Cancer in Patients with a Germline BRCA Mutation. *N Engl J Med* 10(6):523–533 377(
28. Hurvitz SA, Gonçalves A, Rugo HS, Lee KH, Fehrenbacher L, Mina LA, Diab S, Blum JL, Chakrabarti J, Elmeliegy M, DeAnnuntis L, Gauthier E, Czibere A, Tudor IC, Quek RGW, Litton JK, Ettl J. Talazoparib in Patients with a Germline BRCA-Mutated Advanced Breast Cancer: Detailed Safety Analyses from the Phase III EMBRACA Trial. *Oncologist* 2019: 2019 – 0493
29. Heeke AL, Xiu J, Elliott A, Korn WM, Lynce F, Pohlmann PR, Isaacs C, Swain SM, Vidal G, Schwartzberg LS, Tan AR (2020 Nov) Actionable co-alterations in breast tumors with pathogenic mutations in the homologous recombination DNA damage repair pathway. *Breast Cancer Res Treat* 184(2):265–275
30. Pellegrino B, Mateo J, Serra V, Balmaña J (2019) Controversies in oncology: are genomic tests quantifying homologous recombination repair deficiency (HRD) useful for treatment decision making? *ESMO Open*. May 9;4(2):e000480

31. Tung NM, Robson ME, Ventz S, Santa-Maria CA, Nanda R, Marcom PK, Shah PD, Ballinger TJ, Yang ES, Vinayak S, Melisko M, Brufsky A, DeMeo M, Jenkins C, Domchek S, D'Andrea A, Lin NU, Hughes ME, Carey LA, Wagle N, Wulf GM, Krop IE, Wolff AC, Winer EP, Garber JE (2020 Dec) TBCRC 048: Phase II Study of Olaparib for Metastatic Breast Cancer and Mutations in Homologous Recombination-Related Genes. *J Clin Oncol* 20(36):4274–4282 38(
32. VIOLETTE: A randomized phase II study to assess the DNA damage response inhibitors AZD6738 or AZD1775 in combination with olaparib (Ola) versus Ola monotherapy in patients (pts) with metastatic, triple-negative breast cancer (TNBC). *J Clin Oncol*. 2019;37: 15_suppl, TPS1112-TPS1112
33. Dougherty BA, Lai Z, Hodgson DR, Orr MCM, Hawryluk M, Sun J, Yelensky R, Spencer SK, Robertson JD, Ho TW, Fielding A, Ledermann JA, Barrett JC. Biological and clinical evidence for somatic mutations in BRCA1 and BRCA2 as predictive markers for olaparib response in high-grade serous ovarian cancers in the maintenance setting. *Oncotarget*. 2017 Jul 4;8(27):43653–43661. doi: 10.18632/oncotarget.17613
34. Konstantinopoulos PA, Norquist B, Lacchetti C, Armstrong D, Grisham RN, Goodfellow PJ, Kohn EC, Levine DA, Liu JF, Lu KH, Sparacio D, Annunziata CM (2020 Apr) Germline and Somatic Tumor Testing in Epithelial Ovarian Cancer: ASCO Guideline. *J Clin Oncol* 10(11):1222–1245 38(
35. Vikas P, Borchering N, Chennamadhavuni A, Garje R (2020 Apr) Therapeutic Potential of Combining PARP Inhibitor and Immunotherapy in Solid Tumors. *Front Oncol* 28:10:570
36. Sharma P, Rodler E, Barlow WE, Gralow J, Huggins-Puhalla SL, Anders CK, Goldstein LJ, Brown-Glaberman UA, Huynh T, Szyarto CS, Godwin AK, Pathak HB, Swisher EM, Radke MR, Timms KM, Lew DL, Miao J, Pusztai L, Hayes DF, Hortobagyi GN. Results of a phase II randomized trial of cisplatin +/- veliparib in metastatic triple-negative breast cancer (TNBC) and/or germline BRCA-associated breast cancer (SWOG S1416). *Journal of Clinical Oncology* 2020 38:15_suppl, 1001–1001
37. Telli ML, Timms KM, Reid J, Hennessy B, Mills GB, Jensen KC, Szallasi Z, Barry WT, Winer EP, Tung NM, Isakoff SJ, Ryan PD, Greene-Colozzi A, Gutin A, Sangale Z, Iliev D, Neff C, Abkevich V, Jones JT, Lanchbury JS, Hartman AR, Garber JE, Ford JM, Silver DP, Richardson AL. Homologous Recombination Deficiency (HRD) Score Predicts Response to Platinum-Containing Neoadjuvant Chemotherapy in Patients with Triple-Negative Breast Cancer. *Clin Cancer Res*. 2016 Aug 1;22(15):3764-73
38. Clark DF, Maxwell KN, Powers J, Lieberman DB, Ebrahimzadeh J, Long JM, McKenna D, Shah P, Bradbury A, Morrisette JJD, Nathanson KL, Domchek SM. Identification and Confirmation of Potentially Actionable Germline Mutations in Tumor-Only Genomic Sequencing. *JCO Precis Oncol*. 2019;3:PO.19.00076
39. Watkins JA, Irshad S, Grigoriadis A, Tutt AN. Genomic scars as biomarkers of homologous recombination deficiency and drug response in breast and ovarian cancers. *Breast Cancer Res*. 2014 Jun 3;16(3):211
40. Gou R, Dong H, Lin B. *Life Sci*. Application and reflection of genomic scar assays in evaluating the efficacy of platinum salts and PARP inhibitors in cancer therapy. 2020 Nov 15;261:118434
41. Gonçalves A, Bertucci A, Bertucci F (Cancers (Basel). PARP Inhibitors in the Treatment of Early Breast Cancer: The Step Beyond? 2020) May 27;12(6):1378

Tables

Table 1. Clinical presentations of HR-related genes across study group.

	Group1A (n=387)	Group1B (n=25)	Group2 (n=93)	Group3-1 (n=36)	Group3-2 (n=36)*	NonPCR (n=9)	Stage III (n=21)	Stage IV (n=41)
Cased with mutated HR-related genes	74(19.1%)	6(24%)	32(34.4%)	15(41.7%)	12(33.3%)	3(33.3%)	10(47.6%)	12(29.3%)
IHC assay								
HR+/HER2-	60	5	15	8	7	2	7	7
HR+/HER2+	2	1	2	0	2	0	1	1
HR-/HER2+	4	0	5	2	1	0	2	1
HR-/HER2-	8	0	10	5	1	1	0	3

Mutated genes stratified by IHC

(No. of variants,

No. of impacted patients)

HR+/HER2-	HR+/HER2-	HR+/HER2-	HR+/HER2-	HR+/HER2-	HR+/HER2-	HR+/HER2-	HR+/HER2-	HR+/HER2-
ARID1A(18,14)	BRCA2(4,3)	ARID1A(2,2)	ARID1A(4,3)	ARID1A(6,5)	ARID1A(1,1)	ARID1A(3,3)	ARID1A(2,2)	
ATM(7,7)	PALB2(1,1)	BRCA1(1,1)	ATR(1,1)	ATM(13,2)	ATM(1,1)	ATM(2,2)	ATR(1,1)	
ATR(3,3)	PTEN(1,1)	BRCA2(5,3)	BRCA2(1,1)	ATR(19,2)	ATR(4,1)	ATR(1,1)	PALB2(2,1)	
ATR(2,2)	HR+/HER2+	PALB2(8,6)	CHEK1(1,1)	ATR(11,2)	ATR(1,1)	ATR(1,1)	PTEN(4,2)	
BRCA1(3,2)	ARID1A(3,1)	PTEN(4,4)	PALB2(2,2)	BAP1(1,1)	BRCA1(1,1)	BRCA1(1,1)	RAD51C(1,1)	
BRCA2(10,8)	ATM(2,1)	RAD51C(1,1)	PTEN(1,1)	BRCA1(3,2)	BRCA2(2,1)	BRCA2(1,1)	HR+/HER2+	
FANCA(4,4)	ATR(3,1)	HR+/HER2+	RAD51C(1,1)	BRCA2(11,2)	CHEK1(1,1)	FANCA(1,1)	BRCA2(1,1)	
NBN(3,3)	ATR(5,1)	ARID1A(1,1)	HR-/HER2+	CHEK1(3,2)	FANCD2(1,1)	PALB2(1,1)	RAD50(1,1)	
PALB2(14,13)	BAP1(2,1)	BRCA2(1,1)	NBN(1,1)	FANCA(4,2)	MRE11(1,1)	PTEN(3,2)	HR-/HER2+	
PTEN(23,13)	BRCA1(1,1)	HR-/HER2+	PALB2(1,1)	FANCD2(11,2)	PALB2(3,1)	HR+/HER2+	BAP1(1,1)	
RAD50(6,6)	BRCA2(3,1)	ARID1A(1,1)	RAD51C(1,1)	MRE11(5,2)	RAD50(1,1)	ARID1A(1,1)	HR-/HER2-	
RAD51B(1,1)	FANCA(2,1)	ATR(1,1)	HR-/HER2-	NBN(4,2)	RAD51C(2,1)	BRCA2(1,1)	BRCA1(1,1)	
RAD51C(1,1)	FANCD2(1,1)	BRCA1(1,1)	ARID1A(1,1)	PALB2(5,3)	RAD51D(1,1)	RAD50(1,1)	PALB2(1,1)	
HR+/HER2+	MRE11(1,1)	PALB2(1,1)	ATM(1,1)	PTEN(8,4)	HR-/HER2-	HR-/HER2+	PTEN(1,1)	
ARID1A(1,1)	PTEN(2,1)	PTEN(1,1)	ATR(3,2)	RAD50(5,2)	PTEN(1,1)	ARID1A(3,1)	RAD51B(1,1)	
PALB2(1,1)	RAD51D(1,1)	RAD51C(1,1)	ATR(1,1)	RAD51B(5,2)		BAP1(1,1)		
HR-/HER2+		HR-/HER2-	BRCA1(2,2)	RAD51C(1,1)		BRCA1(1,1)		
ARID1A(1,1)		ARID1A(2,1)	BRCA2(3,1)	RAD51D(1,1)		BRCA2(2,1)		
BRCA2(1,1)		ATM(2,1)	PALB2(4,1)	HR+/HER2+		FANCA(1,1)		
FANCA(1,1)		ATR(1,1)	PTEN(2,2)	BRCA1(1,1)		FANCD2(2,1)		
PALB2(1,1)		ATR(2,1)	RAD51B(1,1)	FANCA(1,1)		PTEN(2,1)		
HR-/HER2-		BAP1(1,1)		PALB2(1,1)				
ARID1A(1,1)		BRCA1(2,2)		RAD51C(1,1)				
ATM(1,1)		BRCA2(2,2)		HR-/HER2+				
ATR(1,1)		PALB2(1,1)		BRCA2(1,1)				
ATR(1,1)		PTEN(5,5)		HR-/HER2-				
BRCA1(1,1)				BRCA1(1,1)				
NBN(2,1)				NA				
PALB2(3,3)				ATR(1,1)				
PTEN(1,1)								
RAD50(1,1)								

Figures

Figure 1

Distributions of clinical variables across study groups. The distributions of clinical scenarios were: Group 1A (surgery first, n=387), Group 1B (recurrence within 3 years, n=25), Group 2 (neoadjuvant therapy first, n=93), Group 3-1 (de novo stage IV, n=36), Group 3-2 (recurrence beyond 3 years, n=36), and retrospective biobank cohort (non-PCR, n=9, stage III, n=21 and stage IV, n=41). IHC: immunohistochemistry, m_subtype: molecular subtype.

Figure 2

Mutation maps of BRCA1 (top) BRCA2 (bottom).

Figure 3

Co-occurrence of BRCA1/2. OncoPrinter showed both BRCA1 and BRCA2 altered in 40 (6%) of 648 samples. Patients were arranged at the same order as Fig 1.

Figure 4

OncoPrinter showed HRR genes altered in 107 (17%, Talazoparib Beyond BRCA trail definition, top) and 122 (19%, Heeke et al. definition, bottom) breast cancer samples based on different definition of HRR genes. IHC: immunohistochemistry, m_subtype: molecular subtype.

Figure 5

Chord diagram of mutant HRR genes among Taiwanese breast cancers.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementarymaterials.docx](#)