Predictive value of DNA repair gene expression for response to neoadjuvant chemotherapy in breast cancer

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Abstract

Genome-wide analysis using microarrays has revolutionized breast cancer (BC) research. A substantial body of evidence supports the clinical utility of the 21-gene assay (Oncotype DX) and 70-gene assay (MammaPrint) to predict BC recurrence and the magnitude of benefit from chemotherapy. However, there is currently no genetic tool able to predict chemosensitivity and chemoresistance to neoadjuvant chemotherapy (NACT) during BC treatment. In this study, we explored the predictive value of DNA repair gene expression in the neoadjuvant setting. We selected 98 patients with BC treated with NACT. We assessed DNA repair expression in 98 formalin-fixed, paraffin-embedded core biopsy fragments used at diagnosis and in 32 formalin-fixed, paraffin-embedded post-NACT residual tumors using quantitative reverse transcription-polymerase chain reaction. The following genes were selected: BRCA1, PALB2, RAD51C, BRCA2, ATM, FANCA, MSH2, XPA, ERCC1, PARP1, and SNM1. Of 98 patients, 33 (33.7%) achieved pathologic complete response (pCR). The DNA expression of 2 genes assessed in pre-NACT biopsies (PALB2 and ERCC1) was lower in pCR than in non-pCR patients (P=0.005 and P=0.009, respectively). There was no correlation between molecular subtype and expression of DNA repair genes. The genes BRCA2 (P=0.009), ATM (P=0.004), FANCA (P=0.001), and PARP1 (P=0.011) showed a lower expression in post-NACT residual tumor samples (n=32) than in pre-NACT biopsy samples (n=98). The expression of 2 genes (PALB2 and ERCC1) was lower in pCR patients. These alterations in DNA repair could be considered suitable targets for cancer therapy.

Key words: Breast cancer; Neoadjuvant chemotherapy; Expression of DNA repair genes

Introduction

Breast cancer (BC) is a heterogeneous group of neoplasms in terms of their molecular alterations (1). Specific biological processes, distinct genetic pathways, and different molecular subtypes are associated with different prognosis and sensitivity to treatment (2). Defective DNA repair pathways allow cancer cells to accumulate genomic alterations that contribute to their aggressive phenotype (3). These alterations induce genome instability and promote carcinogenesis steps, cancer progression, and chemoresistance (4,5).

The neoadjuvant setting provides a rich environment for the investigation of therapies and biomarkers (6). Chemotherapy-induced DNA damage is processed by several key pathways that work together to eliminate DNA lesions and maintain genome stability and integrity (4). There is strong emerging evidence that overexpression of DNA repair factors can contribute to resistance to cancer treatment (7).

Genome-wide analysis using microarrays has revolutionized the field of BC research, classifying breast cancer by gene expression profiling (8). In patients with BC, a substantial body of evidence supports the clinical utility of gene expression profiling. For example, the 21-gene assay (Oncotype DX®, USA) and 70-gene assay (MammaPrint®, The Netherlands) predict BC recurrence and the magnitude of benefit from chemotherapy (9,10).
However, there is currently no gene-expression profiling able to predict chemosensitivity and chemoresistance to neoadjuvant chemotherapy (NACT) during BC treatment.

Therefore, the development of specific predictive biomarkers for chemoresistance and chemosensitivity is desirable. In the present study, we explored the predictive value of DNA repair gene expression for response to NACT in BC by evaluating the mRNA expression of 11 selected genes that have a key role in DNA repair mechanisms. In addition, BRCA1, BRCA2, RAD51C, ATM, and PALB2 are tightly correlated with breast cancer and have been associated with breast cancer predisposition, clinicopathological features, and prognosis (11). On the other hand, ERCC1, FANCA, MSH2, XPA, and SNM1A have been well documented as markers of resistance to chemotherapy in solid tumors (7,12–15).

Currently, PARP1 is a therapeutic target in the treatment for patients with BRCA1- or BRCA2-mutated BC (16).

Material and Methods

We retrospectively reviewed the medical records of patients with BC who received NACT from January 2012 to June 2020 at a private clinic and at a General Hospital.

We analyzed data on the patients’ medical history, clinicopathological features, type of surgery, and NACT modality. We excluded patients who had distant metastases at diagnosis.

Pathologic and subtype stratification

We assessed estrogen receptor (ER)/progesterone receptor (PgR) status, human epidermal growth factor receptor 2 (HER2) protein, and Ki-67 antigen with the following primary antibodies: monoclonal antibody (Mab) to ER (Dako, USA, clone EP1, prediluted), MAb to PgR (Dako, clone PgR, prediluted), MIB-1 MAb to Ki-67 antigen (Dako, clone MIB-1, prediluted), and polyclonal antiserum (Biogen, USA, clone SP3, 1/1100 dilution) to HER2 protein. Intense and complete membrane staining in tumor cells qualified for HER2 immunohistochemical (IHC) expression (3+). For this analysis, HER2 scores of 0 and 1+ were considered negative. HER2 IHC 3+ and FISH-amplified tumors were considered positive. All IHC 2+ tumors and for which IHC was not assessable were also tested for gene amplification by FISH. The value of Ki-67 labeling index was divided into low (≤14%) and high (>14%). According to the St. Gallen BC subtype approximations (2), we stratified BC into 5 tumor subtypes: 1) luminal A – ER- and/or PgR-positive, HER2-negative, and low Ki-67 (≤14%); 2) luminal B/HER2-negative – ER- and/or PgR-positive, HER2-negative, and high Ki-67 (>14%); 3) luminal B/HER2-positive – ER- and/or PgR-positive, any Ki-67, and HER2-positive; 4) non-luminal/HER2-positive – ER-negative, PgR-negative, and HER2-positive; and 5) triple negative – ER-negative, PgR-negative, and HER2-negative.

Reverse transcription and gene expression

We selected the following genes for analysis: BRCA1, PALB2, RAD51C, BRCA2, ATM, FANCA, MSH2, XPA, ERCC1, PARP1, and SNM1. The analyses were developed as described by Cronin et al. (17) and Paik et al. (18) for the evaluation of gene expression in formalin-fixed, paraffin-embedded tumor tissue.

After performing hematoxylin and eosin (H&E) staining of a 3-μm slice from the original paraffin blocks by mirroring, a pathologist selected the area of exclusive tumor mass according to the morphological criteria of anatomopathological diagnosis of invasive breast carcinoma in the paraffin block, ensuring the purity of the tumor sample to the detriment of possible contamination of the paraffin sample used for extraction of genetic material. With a surgical blade, we dissected the areas of interest containing representative invasive carcinoma, excluding areas of in situ carcinoma, necrosis, and normal breast tissue. We removed the paraffin by xylene extraction and extracted RNA using the RecoverAll™ Total Nucleic Acid Isolation kit (Invitrogen-ThermoFisher™, USA), according to the manufacturer’s instructions. Immediately after RNA extraction, we performed reverse transcription to obtain cDNA using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems-Thermo Fisher, USA). This material was frozen at −20°C until used in the quantitative reverse transcription-polymerase chain reaction (qRT-PCR) assay.

Before the amplification reaction, we quantified the cDNA with a Qubit® 2.0 fluorometer using the Qubit® dsDNA HS assay (Invitrogen-ThermoFisher™), according to the manufacturer’s instructions. We then performed qRT-PCR with the 7500 Fast Real-Time PCR System using TaqMan® Gene Expression and pre-designed TaqMan® probes (all from Applied Biosystems, USA). Thermocycling conditions included an initial incubation at 50°C for 2 min and at 95°C for 10 min, followed by 45 cycles of 95°C for 15 s and 60°C for 1 min.

Gene expression analyses were performed in the Laboratory of Anatomic Pathology and Molecular Biology (Diagnose Group). The evaluation of the purity and quality of the extracted RNA can be gauged from the good performance in the cDNA quantification and amplification of the chosen reference genes. The cDNA quantification demonstrates the amount of cDNA generated from the extracted RNA following the protocols of the RecoverAll Total Nucleic Acid Isolation Optimized for FFPE Samples kit (Invitrogen-ThermoFisher™). The specifications of the kit inform that the RNA extracted by the methodology is viable for performing RT-PCR and recommend the analysis of small amplicons. Taking this information into account, the quality of the material recovered from the paraffin blocks can be inferred from the amplification of the chosen reference genes GAPDH (Hs03929097_g1), with an amplicon of 58 bp, and ACTB (Hs99999903_m1), with a larger amplicon, 171 bp.
We assessed DNA repair gene expression using pre-designed Taqman® probes (Life Technologies, USA). The expression of each gene was measured in duplicate and then normalized relative to 2 reference genes: ACTB (the gene encoding β-actin) and GAPDH. We used normalization based on reference genes to correct differences arising from variation in RNA quality and total RNA quantity in each assay. We used the mean of 2 reference genes to minimize the risk of normalization bias that can result from variations in the expression of any single reference gene. A reference threshold cycle (Ct) for each tested specimen was defined as the average Ct value of the reference genes. The relative mRNA level of a DNA repair gene within a tissue specimen was defined as $2^{-\Delta Ct}$, where $\Delta Ct = Ct$ (DNA repair gene) – Ct (mean of 2 reference genes).

Identification of the most stable reference genes

The relative qRT-PCR method requires the use of a normalizing gene as an internal control to correct the differences between the compared samples (19). To this end, we selected 3 well-known reference genes from the literature: ACTB, GAPDH, and GUSB. These genes are constitutively expressed across a wide range of tissues and biological conditions and used as reference genes in the Oncotype DX® assay (9). According to the NormFinder (20), geNorm (21), and BestKeeper (22) statistical algorithms and the $\Delta$Ct method (23), ACTB + GAPDH was the best combination when the 3 candidate reference genes were compared.

Briefly, the NormFinder algorithm first merges group division, absolute gene copy number, and the random expression variation (stability value) caused by biological and experimental factors and then ranks the reference genes in order: the lower the stability value, the more reliable the reference gene (20). The geNorm algorithm calculates a stability value, called M, as the average pairwise variation of each reference gene in relation to all other reference genes, thus allowing the elimination of the least stable gene (21). The BestKeeper algorithm computes the average Ct value, standard deviation (SD), and coefficient of variation for each gene, and genes with an SD >1 are considered unsuitable for use as a stable reference gene (22). The comparative $\Delta$Ct method calculates the most stable reference gene from the mean SDs by pairwise comparison of 2 reference genes. An SD <1 indicates stable gene expression (23).

Definitions

Pathologic complete response (pCR) was defined as disappearance of all invasive cancer in the breast after completion of NACT. Overall survival (OS) was calculated from the date of surgery to the date of death from any cause, or the last date of follow-up. Disease-free survival (DFS) was defined as time from surgery to recurrence or death, whichever occurred first, and was censored at the date of last follow-up for those alive without recurrence. Locoregional recurrence (LRR) was defined as local treatment failure (including relapse on the chest wall, local skin, and operative scar) or regional treatment failure (including recurrence of internal mammary, supraclavicular, and ipsilateral axillary nodes).

Statistical analysis

We used SPSS 20.0 (USA) for statistical analyses, and a P-value $\leq 0.05$ was considered statistically significant. Survival curves were calculated by the Kaplan-Meier method, and the differences were assessed by the log-rank test. Gene expressions were analyzed by comparing median values using the Mann-Whitney test. Clinicopathological features were analyzed by the Mann-Whitney test or Fisher’s exact test.

Results

Initially, we selected 147 paraffin blocks containing 108 core biopsies and 39 residual tumors after NACT. Five paraffin blocks containing 6 (5.5%) core biopsies and 4 (10.2%) residual tumor were excluded, as they contained a low density of neoplastic cells in relation to the tumor stroma, making it impossible to select a tumor area with safety. In our study, 7 samples did not reach the quality control level and were excluded (4 core biopsy samples and 3 residual tumor samples). In these samples, the Ct values of the reference genes were very different from the mean Ct values of most samples.

We reviewed the medical records of 98 patients with BC treated with NACT. Median patient age was 46.4 (range 24–77) years. Supplementary Table S1 shows the pCR rate according to clinicopathological features.

Overall, 33 patients (33.7%) achieved pCR. The pCR rate was 33.3% (n=2) for luminal A, 18.7% (n=6) for luminal B/HER2-negative, 36.4% (n=8) for luminal B/HER2-positive, 36.4% (n=4) for non-luminal/HER2-positive, and 48.1% (n=13) for triple negative. The tumor progressed during NACT in 10 patients (10.2%). In univariate analysis, histologic grade (P=0.006), PgR expression (P=0.021), and NACT regimen (P=0.03) were significantly associated with pCR. After a median follow-up of 38.5 months, the 5-year cumulative incidence of LRR was 11.8%. The estimated 5-year DFS was 90.9% in the pCR group and 45.3% in the non-pCR group (P=0.007) (Figure 1). Multivariate analysis by Cox regression showed that patients who presented pCR had better DFS regardless of clinical characteristics related to the molecular subtype, clinical stage, and tumor grade (HR=11.0; 95%CI: 2.51 to 48.22; P=0.001). The estimated 5-year OS was 90.9% and 70.4% in the pCR and non-pCR groups, respectively (P=0.221) (Figure 2). Multivariate analysis by Cox regression showed that patients
who presented pCR had better OS regardless of clinical characteristics related to the molecular subtype, clinical stage, and tumor grade [hazard ratio (HR)=4.85; 95% CI: 1.04 to 22.60; P=0.044].

DNA repair gene expression in pre-NACT biopsies Table 1 summarizes the expression of DNA repair genes in pre-NACT core biopsy fragments according to neoadjuvant response. The DNA expression of 2 genes

| Gene | pCR | non-pCR |
|------|-----|---------|
| 1    | 33  | 65      |
| 2    | 30  | 57      |
| 3    | 22  | 41      |
| 4    | 12  | 28      |
| 5    | 5   | 17      |
| 6    | 3   | 9       |

**Figure 1.** Estimated 5-year disease-free survival by pathologic complete response (pCR) (Kaplan-Meier method). The number of patients at risk at each follow-up time is shown below the graph.

**Figure 2.** Estimated 5-year overall survival by pathologic complete response (pCR) (Kaplan-Meier method). The number of patients at risk at each follow-up time is shown below the graph.
assessed in pre-NACT biopsies (PALB2 and ERCC1) was lower in pCR than in non-pCR patients (P=0.005 and P=0.009, respectively) (Table 1 and Figure 3). After adjustment for tumor grade and molecular subtype, a linear regression model with rank transformation showed that the DNA expression of 2 genes (PALB2 and ERCC1) assessed in pre-NACT biopsies was lower in the pCR group than in the non-pCR group (P=0.014 and P=0.040, respectively).

DNA repair gene expression: pre-NACT biopsies vs post-NACT residual tumor

The genes BRCA2 (P=0.009), ATM (P=0.004), FANCA (P=0.001), and PARP1 (P=0.011) showed a lower expression in post-NACT residual tumor samples (n=32) than in pre-NACT biopsy samples (n=98) (Table 2 and Figure 4).

Discussion

DNA repair mechanism consists of several key pathways working together to eliminate DNA lesions and maintain genome stability and integrity (4). Recently developed multiparameter gene-expression assays, mostly based on the expression of genes involved in different DNA repair pathways, have facilitated the selection of patients who are most likely to benefit from systemic chemotherapy (7). In the present study, the DNA expression of 2 genes (PALB2 and ERCC1) assessed in pre-NACT biopsies was lower in the pCR group than in the non-pCR group (P=0.005 and P=0.009, respectively), suggesting that these genes may be predictive markers for NACT response.

The ERCC1 protein plays an essential role in the nucleotide excision repair pathway. The ERCC1-XPF heterodimer cleaves and facilitates the removal of bulky lesions, such as those induced by platinum-based chemotherapy (24,25). ERCC1 has been well documented as a marker of resistance to chemotherapy in solid tumors other than BC, such as lung, colorectal, head, neck, gastric, bladder, and ovarian cancers (26,27). ERCC1 expression is an excellent predictor of response to chemotherapy regimens based on anthracyclines and taxanes in patients with early- and advanced-stage BC (7). Low ERCC1 expression has been associated with

| Gene   | DNA repair gene expression (percentile) | pCR (n=31) value (x 1000) | Non-pCR (n=63) value (x 1000) | P* |
|--------|----------------------------------------|---------------------------|-------------------------------|----|
| BRCA1  | P50                                    | 4.81                      | 8.78                         | 0.076 |
| PALB2  | P50                                    | 9.71                      | 25.80                        | 0.005 |
| RAD51C | P50                                    | 7.46                      | 4.33                         | 0.183 |
| BRCA2  | P50                                    | 2.79                      | 2.84                         | 0.987 |
| ATM    | P50                                    | 21.83                     | 22.14                        | 0.501 |
| FANCA  | P50                                    | 2.79                      | 1.82                         | 0.896 |
| MSH2   | P50                                    | 27.48                     | 38.76                        | 0.274 |
| XPA    | P50                                    | 12.25                     | 16.13                        | 0.075 |
| ERCC1  | P50                                    | 76.94                     | 186.88                       | 0.009 |
| PARP1  | P50                                    | 9.09                      | 7.09                         | 0.384 |
| SNM1   | P50                                    | 16.13                     | 16.75                        | 0.837 |

NACT: neoadjuvant chemotherapy. *Mann-Whitney test.

Figure 3. DNA repair gene expression of PALB2 and ERCC1 genes in pre-neoadjuvant chemotherapy biopsies by pathologic complete response (pCR). Data are reported as medians (interquartile range). P < 0.05 between groups for the two genes (Mann-Whitney test).
higher pCR rates in the NACT setting and with worse OS in patients with hormone receptor-positive BC (7). A prospective study of patients with metastatic triple-negative BC showed that high ERCC1 expression was associated with worse clinical outcomes for OS and DFS, as well as with a worse response to platinum-based chemotherapy regimens (27).

The PALB2 gene is vital for homologous recombination repair in response to double-stranded DNA breaks. PALB2 has been confirmed as a high-risk BC susceptibility gene in recent large-scale analyses of multigene panel testing, with the odds ratio of PALB2 mutations for BC being comparable to that of BRCA2 mutations (28,29). Patients with BC harboring the PALB2 mutation appear to be more likely to present the triple-negative phenotype, advanced disease stage, and higher Ki-67 levels than patients with other familial or sporadic BC (30). Few studies have correlated PALB2 and chemoresistance. Several deubiquitinases

### Table 2. DNA repair gene expression: pre-NACT biopsies vs post-NACT residual tumor.

| Gene   | DNA repair gene expression (percentile) | Core biopsy (n=98) value (× 1000) | Residual tumor (n=32) value (× 1000) | P*   |
|--------|----------------------------------------|-----------------------------------|-------------------------------------|------|
| BRCA1  | P50                                    | 7.66                              | 6.69                                | 0.131|
| PALB2  | P50                                    | 17.07                             | 24.09                               | 0.166|
| RAD51C | P50                                    | 5.42                              | 6.26                                | 0.248|
| BRCA2  | P50                                    | 2.79                              | 0.16                                | 0.009|
| ATM    | P50                                    | 21.83                             | 17.11                               | 0.004|
| FANCA  | P50                                    | 1.85                              | 0.46                                | 0.001|
| MSH2   | P50                                    | 33.08                             | 42.98                               | 0.218|
| XPA    | P50                                    | 14.36                             | 24.31                               | 0.395|
| ERCC1  | P50                                    | 131.93                            | 324.85                              | 0.158|
| PARP1  | P50                                    | 7.12                              | 2.55                                | 0.011|
| SNM1   | P50                                    | 16.49                             | 11.51                               | 0.159|

NACT: neoadjuvant chemotherapy. *Mann-Whitney test.

![Figure 4. DNA expression of repair genes in post-NACT residual tumors and pre-NACT biopsies. NACT: neoadjuvant chemotherapy. Data are reported as medians (interquartile range). P < 0.05 between groups for the four genes (Mann-Whitney test).](image)
have been implicated as key players in DNA damage repair through homologous recombination. In lung adenocarcinoma, USP22, a deubiquitinase highly overexpressed in multiple cancer types, has been shown to modulate PALB2 levels through its C-terminal WD40 domain to promote chemo-resistance (31). In metastatic BC, germline PALB2 mutation has been associated with improved disease response to platinum-based chemotherapy (32).

Despite the scarce literature on ERCC1 and PALB2 in NACT for BC, our results are consistent with the available data. This highlights the importance of further exploring these DNA repair pathways in the neoadjuvant BC setting. Recently, the I-SPY 2 trial, an adaptive clinical trial platform, was designed to improve outcomes in high-risk breast cancer patients by testing new drugs in the neoadjuvant setting. This study used clinical biomarkers to classify breast cancer into 10 subtypes, allowing individualized patient assignment to therapy arms and maximizing treatment effects (33). The development of specific predictive biomarkers represents the future of breast cancer management and leads to more personalized treatments (33).

In the present study, when comparing the expression of the 11 selected genes between pre-NACT biopsy samples (n=98) and post-NACT residual tumor samples (n=32), we found a lower expression of the genes BRCA2 (P=0.009), ATM (P=0.004), FANCA (P=0.001), and PARP1 (P=0.011) in residual tumor samples. The inactivation of these genes may be directly related to chemoresistance mechanisms, in which cells activate the repair mechanism for damage elimination or proceed toward apoptosis in response to DNA damage by NACT (34). Further studies focusing on the cellular DNA repair mechanism pathways are needed to expand our understanding of BC etiology and to develop therapies specifically targeting the defective pathway in patients with BC (4). This strategy is well established in the treatment of ovarian cancer with PARP1 inhibitors. PARP1 is a promising treatment target in BRCA-deficient carcinomas. Homologous recombination-deficient BRCA-mutant carcinomas, which rely on PARP1-base excision repair for survival, are highly sensitive to PARP1 inhibitors through the mechanism of synthetic lethality (35,36).

The strengths of our study include the comprehensive nature of the registry database containing patient characteristics, clinicopathological features, surgery description, adjuvant therapies, and complete ascertainment of patient status at regular follow-up intervals. Another important point is that all gene expression analyses were conducted in a reference center by experts in the field. However, this study also has limitations, including its retrospective nature, small sample size, and heterogeneous population. Furthermore, the high pCR rate for the luminal A subtype may be due to the small number of patients.

Conclusion
In the era of genomics, treatment should be tailored to the individual patient. The present study showed that PALB2 and ERCC1 expressions, assessed in pre-NACT biopsies, were lower in pCR patients. In addition, BRCA2, ATM, FANCA, and PARP1 expressions were lower in post-NACT residual tumor samples than in pre-NACT biopsies. The use of recently developed multiparameter gene-expression assays, based on the expression of genes involved in different DNA repair pathways, should be further explored in future studies, as they may facilitate the selection of patients most likely to benefit from NACT.

Supplementary Material
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References
1. Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. *Nature* 2000; 406: 747–752, doi: 10.1038/35021093.
2. Goldhirsh A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thürlimann B, et al. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. *Ann Oncol* 2013; 24: 2206–2223, doi: 10.1093/annonc/mdt303.
3. Dietlein F, Thelen L, Reinhardt HC. Cancer-specific defects in DNA repair pathways as targets for personalized therapeutic approaches. *Trends Genet* 2014; 30: 326–339, doi: 10.1016/j.tig.2014.06.003.
4. Majidinia M, Yousefi B. DNA repair and damage pathways in breast cancer development and therapy. *DNA Repair (Amst)* 2017; 54: 22–29, doi: 10.1016/j.dnarep.2017.03.009.
5. Motegi A, Masutani M, Yoshioka KI, Bessho T. Aberrations in DNA repair pathways in cancer and therapeutic significances. *Semin Cancer Biol* 2019; 58: 29–46, doi: 10.1016/j.semcancer.2019.02.005.
6. Haddad TC, Goetz MP. Landscape of neoadjuvant therapy for breast cancer. *Ann Surg Oncol* 2015; 22: 1408–1415, doi: 10.1245/s10434-015-4405-7.
7. Abdel-Fatah TMA, Ali R, Sadiq M, Moseley PM, mesquita KA, Ball G, et al. ERCC1 Is a predictor of anthracycline resistance and taxane sensitivity in early stage or locally
advanced breast cancers. Cancers (Basel) 2019; 11, doi: 10.3390/cancers11081149.

8. Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci USA 2001; 98: 10869–10874, doi: 10.1073/pnas.191367098.

9. Sparano JA, Gray RJ, Ravdin PM, Makower DF, Pritchard KI, Albain KS, et al. Clinical and genomic risk to guide the use of adjuvant therapy for breast cancer. N Engl J Med 2019; 380: 2395–2405, doi: 10.1056/NEJMoa1904819.

10. Audeh W, Blumencranz L, Kling H, Trivedi H, Srkalovic G. A population-based study of genes previously implicated in breast cancer. N Engl J Med 2020; 384: 440–451, doi: 10.1056/NEJMo2005936.

11. Hu C, Hart SN, Gnanaoilvou R, Huang H, Lee KY, Na J, et al. A germline FANCA alteration that is associated with increased sensitivity to DNA damaging agents. Cold Spring Harb Mol Case Stud 2017; 3: a001487, doi: 10.1101/mcs.a001487.

12. Wilkes DC, Sailer V, Xue H, Cheng H, Collins CC, Gleave M, et al. ERCC1-XPF expression in epithelial ovarian cancers: role in chemotherapy resistance. Asian Pac J Cancer Prev 2017; 18: 507–513, doi: 10.22034/APJCP.2017.18.2.507.

13. Malik SS, Masood N, Asif M, Ahmed P, Shah ZU, Khan JS. Expression analysis of MLH1 and MSH2 in breast cancer. Curr Probi Cancer 2019; 43: 97–105, doi: 10.1016/j.curprobcancer.2018.08.001.

14. Borszkiewicz Pulzóva, L., Ward TA, Chovanec M. XPA: dna repair protein of significant clinical importance. Int J Mol Sci 2020; 21: 2182, doi: 10.3390/ijms21062182.

15. Baddock HT, Yosaatmadja Y, Newman JA, Schofield CJ, Gileadi O, McHugh PJ. The SNM1A DNA repair nuclease. DNA Repair (Amst) 2020; 95: 102941, doi: 10.1016/j.dnarep.2020.102941.

16. Tutt ANJ, Garber JE, Kaufman B, Viale G, Fumagalli D, Rastogi P, et al. Adjuvant Olaparib for Patients with BRCA1- or BRCA2-mutated breast cancer. N Engl J Med 2021; 384: 2394–2405, doi: 10.1056/NEJMo2105215.

17. Cronin M, Pho M, Dutta D, Stephans JC, Shaks, Kieber MC, et al. Measurement of gene expression in archival paraffin-embedded tissues: development and performance of a 92-gene reverse transcriptase-polymerase chain reaction assay. Am J Pathol 2004; 164: 35–42, doi: 10.1016/S0002-9440(10)63093-3.

18. Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med 2004; 351: 2817–2826, doi: 10.1056/NEJMoa041588.

19. Kozera B, Rapacz M. Reference genes in real-time PCR. J Appl Genet 2013; 54: 391–406, doi: 10.1007/s13353-013-0173-x.

20. Andersen CL, Jensen JL, Omtoft TF. Normalization of real-time quantitative reverse transcription-PCR data: a model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. Cancer Res 2004; 64: 5245–5250, doi: 10.1158/0008-5472.CAN-04-0496.

21. Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol 2002; 3: RESEARCH0034, doi: 10.1186/gb-2002-3-7-research0034.

22. Pfaff MW, Tichopad A, Prgomet C, Neuvians TP. Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper – Excel-based tool using pairwise correlations. Biotechnol Lett 2004; 26: 509–515, doi: 10.1023/B:BILE.0000019559.84305.47.

23. Silver N, Best S, Jiang J, Thein SL. Selection of housekeeping genes for gene expression studies in human reticulocytes using real-time PCR. BMC Mol Biol 2006; 7: 33, doi: 10.1186/1471-2199-7-33.

24. Simon GR, Ismail-Khan R, Bepler G. Nuclear excision repair-based personalized therapy for non-small cell lung cancer: from hypothesis to reality. Int J Biochem Cell Biol 2007; 39: 1318–1328, doi: 10.1016/j.biocel.2007.05.006.

25. Fousteri M, Mullenders LH. Transcription-coupled nucleotide excision repair in mammalian cells: molecular mechanisms and biological effects. Cell Res 2008; 18: 73–84, doi: 10.1010/cr.2008.6.

26. Mesquita KA, Alabduallah M, Griffin M, Toss MS, Fatah TMAA, Abity A, et al. ERCC1-XPF deficiency is a predictor of olaparib induced synthetic lethality and platinum sensitivity in epithelial ovarian cancers. Gynecol Oncol 2019; 153: 416–424, doi: 10.1016/j.ygyno.2019.02.014.

27. EL Baiomy MA, El Kashef WF. ERCC1 expression in advanced breast cancers. Cancers (Basel) 2019; 11, doi: 10.3390/cancers11081149.
chemotherapy on pathologic complete response in women with early-stage breast cancer an analysis of the ongoing phase 2 adaptively randomized I-SPY2 trial. *JAMA Oncol* 2020; 6: 676–684, doi: 10.1001/jamaoncol.2019.6650.

34. Tian H, Gao Z, Li H, Zhang B, Wang G, Zhang Q, et al. DNA damage response—a double-edged sword in cancer prevention and cancer therapy. *Cancer Lett* 2015; 358: 8–16, doi: 10.1016/j.canlet.2014.12.038.

35. D’Andrea AD. Mechanisms of PARP inhibitor sensitivity and resistance. *DNA Repair (Amst)* 2018; 71: 172–176, doi: 10.1016/j.dnarep.2018.08.021.

36. Yang Y, Du N, Xie L, Jiang J, Mo J, Hong J, et al. The efficacy and safety of the addition of poly ADP-ribose polymerase (PARP) inhibitors to therapy for ovarian cancer: a systematic review and meta-analysis. *World J Surg Oncol* 2020; 18: 151, doi: 10.1186/s12957-020-01931-7.