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Immunophenotypic predictive profiling of *BRCA1*-associated breast cancer

Pawel Domagala · Tomasz Huzarski · Jan Lubinski ·
Karol Gugala · Wenancjusz Domagala

Abstract The immunophenotypic predictive profile of *BRCA1*-associated cancers including major predictive markers, i.e., PARP-1, EGFR, c-kit, HER-2, and steroid hormones (ER/PR) that may have therapeutic relevance has not yet been reported in a comprehensive study. Using immunohistochemistry, we examined the expression of these proteins in a large cohort of *BRCA1*-associated breast cancers. PARP-1 immunoreactivity was found in 81.9%, EGFR in 43.6%, ER/PR in 17.9%, c-kit in 14.7%, and overexpression of HER-2 in 3.6% of cancers. For all markers studied, 8.2% of tumors were negative. Expression of only one predictive marker was found in 29.7% of cancers, and most frequently, it was PARP-1 (20.8%). In 62.1% of tumors, more than one predictive marker was expressed: PARP-1 and EGFR in 30.4%, PARP-1, and hormone receptors in 13.3% and PARP-1 with c-kit in 7.5% of all tumors. Coexpression of two or more other predictive markers was rare. There were significant differences in the median age at diagnosis of *BRCA1*-associated cancer between patients with ER+ vs. ER− and grades 1–2 vs.

grade 3 tumors. These results demonstrate that *BRCA1*-associated cancers differ with respect to expression of proteins that are regarded as targets for specific therapies and that 92% of patients with *BRCA1*-associated cancers may benefit from one or several options for specific therapy (in addition to DNA damaging agents, e.g., cisplatin). About 8% of cancers which do not express therapeutic target proteins may not respond to such therapies. Knowledge of the immunophenotypic predictive profile may help with the recruitment of patients for trials of targeted therapies.

Keywords Breast cancer · *BRCA1* · Estrogen receptor · PARP-1 · EGFR · c-kit

P. Domagala · T. Huzarski · J. Lubinski
Department of Genetics and Pathology, International Hereditary Cancer Center, Pomeranian Medical University,
Polabska 4,
70-115 Szczecin, Poland

K. Gugala
Department of Pathology, Regional Oncology Hospital,
Zolnierska 18,
10-561 Olsztyn, Poland

W. Domagala (✉)
Department of Pathology, Pomeranian Medical University,
Unii Lubelskiej 1,
71-242 Szczecin, Poland
e-mail: sekrpato@ams.edu.pl

Introduction

Mutations in either of the two breast cancer susceptibility genes *BRCA1* and *BRCA2* account for 2–3% of all breast cancers, and they confer a lifetime risk of breast cancer of 60–85% [1]. *BRCA1* is essential for the repair of DNA double-strand breaks through the process of homologous recombination. In the absence of functional *BRCA1,* DNA defects may either be repaired by other mechanisms that are error-prone or may remain unrepaired. Such *BRCA* dysfunction constitutes the basis of some new targeted therapeutic strategies [2, 3].

With a better understanding of the molecular pathways that control cancer development and progression, new therapeutic agents designed to target specific breast cancer populations, such as poly(ADP-ribose)polymerase-1 (PARP-1) inhibitors, epidermal growth factor receptor (EGFR) antagonists, or transmembrane tyrosine kinase c-kit (CD117) inhibitors, are being tested in patients with

breast cancer and, in particular, in patients with *BRCA1*-associated cancer [3, 4]. However, despite the significant progress that has been made, a variable percentage of patients have been resistant to such therapies. One possible reason may be that resistant tumors lack the expression of proteins that serve as targets for such specific therapies. In this context, we studied in this report immunohistochemical expression of PARP-1, EGFR, estrogen receptor (ER), progesterone receptor (PR), c-kit, and HER-2 in tissue microarrays of a large series of *BRCA1*-associated breast carcinomas in order to reveal subpopulations of *BRCA1*-associated cancers that exhibit predictive immunophenotypes, i.e., cancers that could respond to specific targeted therapies. The expression of common predictive markers such as ER, PR, HER-2, and EGFR has been reported in series of *BRCA1*-associated breast carcinomas [5–9]; however, a comprehensive study including these proteins with PARP-1 and c-kit has not been published.

Materials and methods

Patients This study included 140 consecutive breast cancer patients with *BRCA1* mutations (median age, 46.0 years; range, 23–71 years) diagnosed at the West Pomeranian Oncology Center in Szczecin (from 1996 to 2009) and at the Regional Oncology Hospital in Olsztyn (from 1997 to 2007). Patients did not receive endocrine therapy, chemotherapy, or radiotherapy before surgery. All cases were unselected for family history. The study was approved by the local ethics committee.

Genotyping Patients were invited to participate either during hospital stays or through mailed invitations. During the interview, the goals of the study were explained, informed consent was obtained, genetic counseling was given, and a blood sample was taken for DNA analysis. *BRCA1* genetic testing was conducted at the Department of Genetics and Pathology, Pomeranian Medical University, Szczecin. Genomic DNA was prepared from 5–10 ml of peripheral blood. Mutation analysis for the common Polish mutations was performed as described previously [10]. In brief, there are three common founder mutations in *BRCA1* in Poland. The 4153delA and 5382insC mutations were detected using a multiplex-specific polymerase chain reaction (PCR) assay. The third mutation (C61G) generates a novel restriction enzyme site in exon 5. This mutation is detected after digesting amplified DNA with AvaII restriction enzyme. To visualize the different *BRCA1* alleles, the PCR products were subjected to electrophoresis in a 1.5% agarose gel and stained with ethidium bromide. To avoid false results in all reactions, positive and negative controls (without DNA) were used. DNA testing results indicating

the presence of mutations were confirmed by sequencing of material from a second blood sample obtained on a different day.

Tumor pathology Pathology review was conducted at the Department of Pathology, Pomeranian Medical University in Szczecin by two pathologists (PD and TH) associated with the study. In cases where there was disagreement, consensus was reached by consultation with a third reviewer (WD). Only first primary invasive breast cancers were included. Representative histological slides organized according to an assigned random number were evaluated to confirm the diagnosis of breast cancer type and classified according to the Elston–Ellis histological grade [11].

Tissue microarray construction We collected all available paraffin blocks containing enough tumor tissue from primary breast cancers. Two different regions of tumors in the area of the outer invasive margin of cancer were identified and marked on hematoxylin and eosin-stained sections. Sections were matched to their corresponding wax blocks (the donor blocks), and two 0.6-mm-diameter cores of the tumor were removed from these donor blocks and inserted into the recipient master block using a tissue microarrayer (Beecher Instruments, Silver Spring, MD). The recipient block was cut, and sections were transferred onto adhesive slides.

Immunohistochemistry and fluorescent in situ hybridization Slides were deparaffinized, rehydrated, and immersed in antigen retrieval buffer at pH 6.0 (PARP-1 and c-kit) or pH 9.0 (ER and PR). Heat-induced antigen retrieval was performed in a water bath at 98°C for 20 min (PARP-1) or a pressure cooker at 120°C for 3 min (ER, PR, and c-kit). The following monoclonal antibodies were used: anti-PARP-1 (clone F-2, dilution 1:500; Santa Cruz Biotechnology, Santa Cruz, CA), anti-estrogen receptor (clone 1D5, dilution 1:50; Dako, Glostrup, Denmark), and anti-progesterone receptor (clone PgR 636, dilution 1:50; Dako). We performed preliminary experiments with breast cancer tissue microarray to determine the optimal PARP-1 antibody dilutions which would give the strongest nuclear-specific staining with minimal background. Of several dilutions tested, the dilution 1:500 proved to be the best. Expression of HER-2 and c-kit was tested using the HercepTest kit (Dako) and c-kit pharmDx kit (Dako), respectively, according to the manufacturer's instructions. EGFR staining was performed using the EGFR pharmDx kit (Dako) with incubation with proteinase K for 5 min for enzymatic antigen retrieval. Slides were incubated with the primary antibodies for 30 min at room temperature and immunostained using the EnVision+ kit (Dako). The reaction was developed with a diaminobenzidine substrate–chromogen solution, and slides were counter-

stained with hematoxylin. Appropriate positive and negative controls were included. Normal mouse immunoglobulins were substituted for primary antibody as negative controls. Sections of pharyngeal tonsil served as external positive controls for PARP-1. Strong PARP-1 immunostaining was seen in the tonsil's lymphocytes. PARP-1-positive stromal lymphocytes in breast cancers served as additional built-in positive control. Cases with HER-2 staining of 2+ were further evaluated by fluorescent in situ hybridization for *HER-2* gene amplification. In this assay, slides were hybridized with probes to LSI HER-2/neu and CEP17 with the PathVysion HER-2 DNA Probe Kit (Abbott Laboratories, Abbott Park, IL) according to the manufacturer's instructions. Fluorescent in situ hybridization with an amplification ratio >2.2 was considered positive.

Immunohistochemistry scoring Tumor cores were independently assessed by two observers (PD and WD) who were blinded to mutation status and clinicopathological data. In cases of disagreement, the result was reached by consensus. Tumors with lost cores or insufficient tumor tissue in the cores were excluded from the analysis. Of the three most frequently applied scoring systems (intensity score, pattern score, or both combined), we used the multiplicative quickscore method because it seemed to be the most reliable and proved to be useful and reproducible [12]. This system accounts for both the intensity and the extent of cell staining. Briefly, the proportion of positive cells was estimated and given a score on a scale from 1 to 6 (1=1% to 4%, 2=5% to 19%, 3=20% to 39%, 4=40% to 59%, 5= 60% to 79%, and 6=80% to 100%). The average intensity of the positively staining of cells was given a score from 0 to 3 (0=no staining, 1=weak, 2=intermediate, and 3= strong staining). A final score was then calculated by multiplying the percentage score by the intensity score, to yield a minimum value of zero and a maximum value of 18. Based on the final score, nuclear PARP-1 expression was graded as low (0–9, further referred as negative) or high (10–18, further referred as positive). Tumors were considered as HER-2 positive if scored as 3+ or 2+ with amplification tested by FISH [13]. ER and PR were considered positive if staining was detected in ≥1% of nuclei [14]. There are no commonly accepted cut-off points reported for EGFR and c-kit. For scoring of samples stained with EGFR, only unequivocally membranous staining in ≥1% of tumor cells was applied to define protein positivity according to the Dako criteria in the pharmDx kit instructions. For c-kit cytoplasmic and/or membranous staining, ≥10% of tumor cells was counted as positive [15].

Statistics Analysis of differences in distributions of study markers expression between the two groups of patients was performed using the Mann–Whitney U test, and differences between more than two groups were evaluated by the Kruskal–Wallis test. The chi-square contingency test for categorical variables was used to determine differences between groups. Spearman's nonparametric correlation test was used for correlative analysis. All reported p values were two sided. For all statistical analyses, a p value <0.05 was considered significant. Statistical analyses were performed using GraphPad Prism 5.0 software (San Diego, CA).

Results

Table 1 lists the clinicopathological details of 140 tumors and patients with *BRCA1* mutations. PARP-1 immunoreactivity (Fig. 1a, b) was found in 81.9% of cancers, EGFR overexpression (Fig. 1e, f) in 43.6%, hormone receptor (ER/PR) immunoreactivity in 17.9%, c-kit (Fig. 1g, h) in 14.7%, and overexpression of HER-2 in only 3.6% of cancers (Table 2). Eleven (8.2%) tumors were negative for all predictive markers studied (pentanegative). Five of the latter were atypical medullary, four were ductal grade 3, one was medullary, and one was metaplastic carcinoma. It is worth noting that 18.1% of *BRCA1*-associated cancers were PARP-1 negative (Fig. 1c, d; 20.7% in the triple-negative subgroup). Of these, 44% were pentanegative. Expression of only one predictive marker was found in 29.7% of *BRCA1*-associated cancers, and most frequently, it was the expression of PARP-1 (20.8%) followed by EGFR (6.7%). In 62.1% of tumors, more than one predictive marker was expressed, and a coexpression of PARP-1 and EGFR constituted 30.4% of all tumors (Table 3). Coexpression of PARP-1 and hormone receptors was found in 13.3% of all cancers and PARP-1 with c-kit in 7.5%. Coexpression of two or more other predictive markers was a rare event. In one tumor, a coexpression was seen of all markers studied with the exception of EGFR (Table 3). There were no statistically significant differences between the frequencies of *BRCA1*-associated breast cancers expressing only one marker, two markers, or more than two markers in the group of young patients (<50 years of age) vs. older patients ($p=0.89$). A cumulative frequency distribution of PARP-1, EGFR, ER, PR, and c-kit showed that PARP-1 was the marker with the highest frequency of tumors with very high score (Fig. 2). A statistically significant positive correlation between the expression of ER and PR ($r=0.81$, $p<0.0001$) and negative correlation between the expression of ER and EGFR ($r=-0.26$, $p=0.002$) as well as PR and EGFR ($r=-0.24$, $p<0.005$) were found. There were significant differences in the median age at the diagnosis of *BRCA1*-associated cancers between patients with ER+ vs. ER− ($p=0.01$) as well as grades 1–2 vs. grade 3 tumors ($p=0.02$, Fig. 3). No statistically

Table 1 Clinicopathological characteristics of 140 *BRCA1*-associated breast carcinomas

	Number	Percentage
Age		
<50	98	70.0
≥50	42	30.0
Histological type		
Ductal	83	59.3
Atypical medullary	32	22.8
Medullary	17	12.1
Metaplastic	4	2.9
Other	4	2.9
Histological grade		
1	2	1.5
2	11	7.8
3	127	90.7
Nodal status (*n*=136)		
Positive	44	32.4
Negative	92	67.6
Tumor size (*n*=131)		
≤2	58	44.3
>2	73	55.7
Bilateral		
Yes	29	20.7
No	111	79.3
Triple negative		
Yes	112	80.0
No	28	20.0
Mutation		
5382insC	90	64.3
C61G	34	24.3
4153delA	16	11.4

significant differences were noted for PR (*p*=0.14) or the other markers studied.

Discussion

In the present study, we report a five marker immunophenotypic predictive profile of *BRCA1*-associated cancers including PARP-1, EGFR, c-kit, HER-2, and steroid hormones (ER/PR). ER and PR are considered one predictive marker because expression of either one predicts response to hormonal therapy. Recently, it has become obvious that classical pathologic features used to predict a treatment response are insufficient as a predictive tool. Patients with *BRCA1*-associated cancers in the same stage of the disease may exhibit variable responses to therapy, hence the need to explain these differences and reveal the

underlying molecular mechanisms. Although there are several ongoing clinical trials aimed at demonstrating the value of targeted therapies in patients with *BRCA1*-associated breast cancers [2, 3], no comprehensive study has been published examining the expression in tumor cells of major proteins against which those therapies are directed.

Our results indicate that *BRCA1*-associated cancers differ with respect to expression of major proteins that are regarded as targets for specific therapies. A variable percentage of *BRCA1*-associated cancers expressed each one of the five major predictive markers, ranging from 82% of PARP-1 positive cancers to only 3.6% of HER-2 positive tumors. These findings have potential therapeutic implications. It has been recognized that cisplatin compounds may effectively target *BRCA1*-associated cancers [16] because *BRCA1*-deficient cells have an impaired ability to repair DNA damage via homologous recombination [17]. Our results indicate that in addition to cisplatin, roughly 30% of *BRCA1*-associated patients may have one option of specific targeted therapy while the remaining 62% of patients with tumors which expressed two or more predictive markers may have several options that should be judiciously considered by their physicians in order to apply the most effective treatment. Furthermore, it seems that about 82% of patients with *BRCA1*-associated cancers that express PARP-1 protein will most likely respond to PARP-1 inhibitor therapy, 44% expressing EGFR may respond to EGFR antagonists, approximately 18% may respond to hormone therapy, and 15% may respond to c-kit inhibitors (e.g., imatinib), but only about 4% may respond to anti-HER-2 therapy (e.g., trastuzumab). However, about 8% may not respond to any of the abovementioned targeted therapies because their tumors do not express proteins that are therapeutic targets (pentanegative, i.e., PARP-1, HER-2, EGFR, c-kit, and steroid hormone receptor negative tumors).

In this report, we identified a subpopulation of *BRCA1*-associated breast cancers characterized by low or null expression of PARP-1 protein. This subpopulation has two pathways of DNA repair inactive or severely compromised, i.e., base excision repair pathway and homologous recombination pathway. PARP-1 is a nuclear enzyme that plays an important role in DNA damage repair through the base excision single-strand repair pathway [18]. When PARP-1 is inhibited, homologous recombination repair is used in response to DNA damage. However, *BRCA*-associated cancers cannot use the homologous recombination pathway; hence, PARP inhibition leads to tumor cells death. This novel concept of treating *BRCA*-associated cancer by causing so-called "synthetic lethality" [19, 20] led to clinical trials of PARP-1 inhibitors in breast cancer [3]. Several active clinical trials involve *BRCA*-associated or triple-negative (ER−, PR−, HER-2−) breast carcinomas.

Fig. 1 Expression of PARP-1, EGFR, and c-kit in representative cores of *BRCA1*-associated breast carcinoma. **a**, **c** Fragment of a tissue microarray with a two neighboring cores from different cancers. **a** PARP-1 expression in the nuclei of cancer cells. **b** Marked fragment of the core from **a** at high magnification showing PARP-1-positive (*brown*) nuclei of cancer cells and some lymphocytes. Mitosis (*arrow*) with cytoplasmic PARP-1 immunoreactivity. **c** PARP-1 negative cancer. **d** Marked fragment of the core from **c** at high magnification showing lack of PARP-1 immunoreactivity. **e** EGFR expression. **f** Marked fragment of the core from **e** showing membranous EGFR staining. **g** c-kit expression. **h** Marked fragment of the core from **g** showing cytoplasmic and membranous expression of c-kit
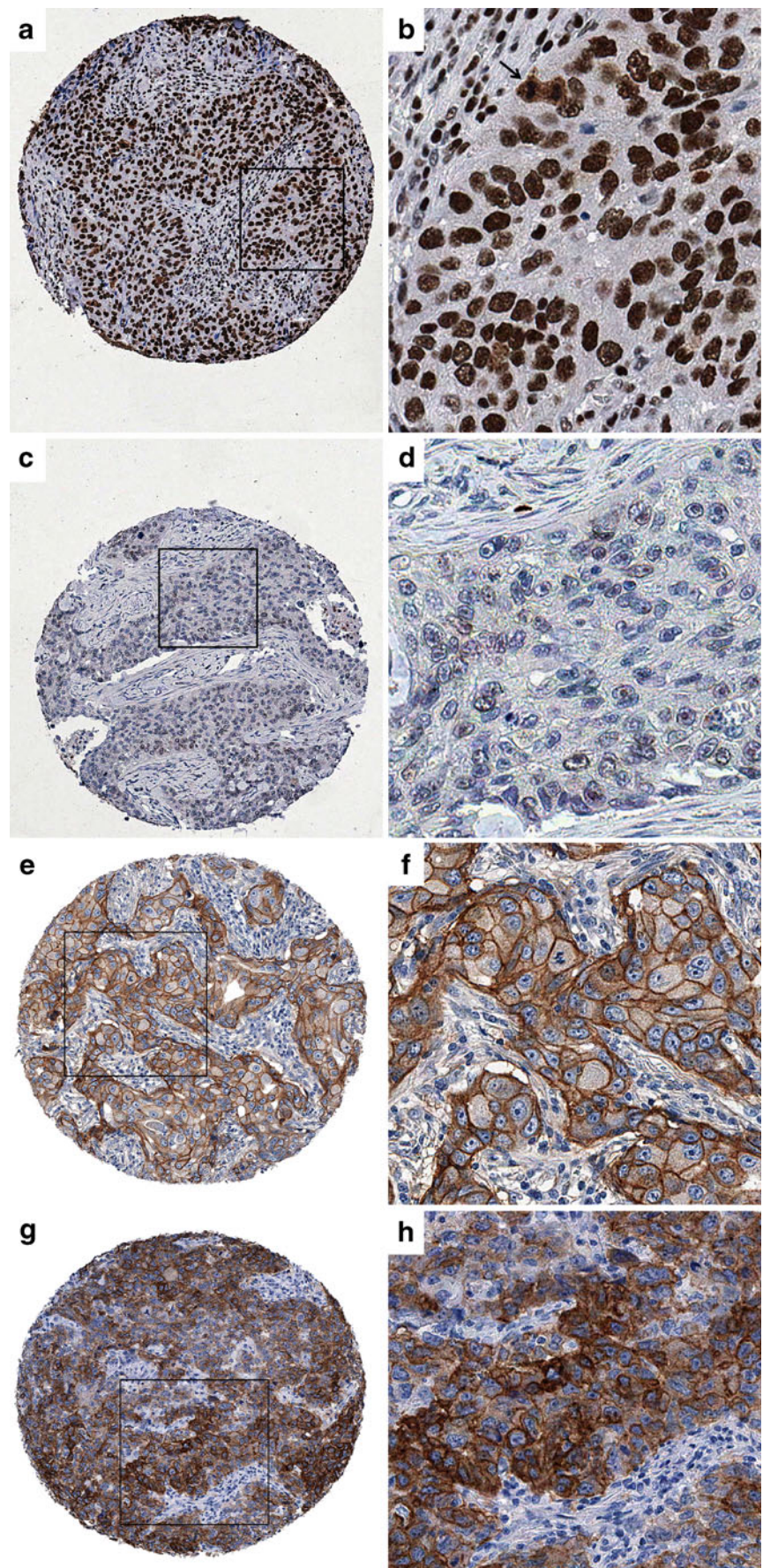
Table 2 Expression of ER/PR, HER-2, EGFR, PARP-1, and c-kit in *BRCA1*-associated breast carcinomas

|	Number[a]	Positive (%)	Negative (%)
PARP-1	138	113 (81.9)	25 (18.1)
EGFR	140	61 (43.6)	79 (56.4)
ER and/or PR[b]	140	25 (17.9)	115 (82.1)
c-kit	136	20 (14.7)	116 (85.3)
HER-2	140	5 (3.6)	135 (96.4)

[a] Tumors with lost cores or insufficient tumor tissue in cores were excluded from the analysis

[b] Only one case was PR positive and ER negative

However, the success of PARP inhibitor therapy may depend not only on the constitutional genetic background (responsible for impairment of DNA repair mechanisms) but also on the level of PARP-1 protein expression in tumor cells. A dose-dependent clinical response to PARP inhibitor therapy in phase 1 [21] and phase 2 [22] studies and less abundant PARP-1 in MCF-7 cells that are resistant to mitoxantrone and etoposide than in cells susceptible to these drugs [23] suggest the therapeutic importance of the level of PARP-1 expression in tumor cells. Our results indicate that approximately 18% of *BRCA1*-associated breast cancers may be refractory to PARP-1 inhibitor therapy because of lack or low expression of PARP-1.

Table 3 Number (%) of *BRCA1*-associated cancers with the expression of one or more or null predictive markers

	Number	Percentage
5-Negative	11	8.2
One marker		
PARP-1+	28	20.8
EGFR+	9	6.7
c-kit+	2	1.5
ER/PR+	1	0.7
Coexpression of two markers		
PARP-1+, EGFR+	41	30.4
PARP-1+, ER/PR+	18	13.3
PARP-1+, c-kit+	10	7.5
PARP-1+, HER-2+	3	2.2
EGFR+, ER/PR+	1	0.7
EGFR+, c-kit+	1	0.7
Coexpression of more than two markers		
PARP-1+, EGFR+, c-kit+	6	4.4
PARP-1+, EGFR+, ER/PR+	2	1.5
PARP-1+, ER/PR+, HER-2+	1	0.7
PARP-1+, ER/PR+, c-kit+, HER-2+	1	0.7
Total	135	100
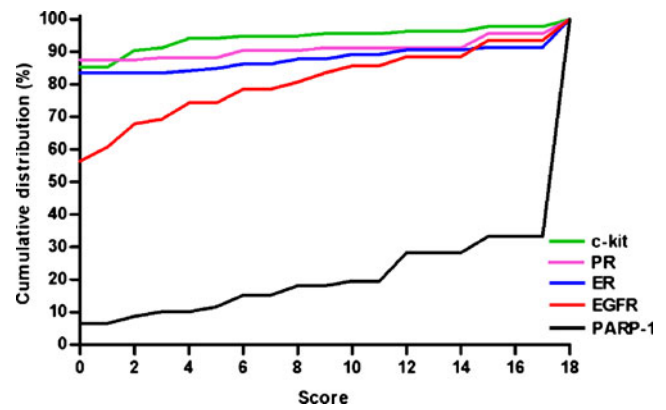
Fig. 2 Cumulative frequency distribution of EGFR, c-kit, PARP-1, ER, and PR in the whole cohort. PARP-1 shows the highest frequency of cases with very high scores

The results of studies on PARP$^{-/-}$ mice and cell lines indicate that they may be extremely sensitive to genotoxic agents such as alkylating agents and γ-rays [24, 25]. This may be especially important in treatment of pentanegative tumors (44% of all tumors with lack or low expression of PARP-1) because they lack protein targets for major targeted therapies.

Our data suggest that about 55% of *BRCA1*-associated cancers may not respond to anti-EGFR therapy because they show null or very low expression of this protein. Equally important seems to be the result indicating that the vast majority of *BRCA1*-associated cancers may be relatively resistant to hormonal therapy as well as to anti-c-kit and anti-HER-2 therapies. Although one has to take into
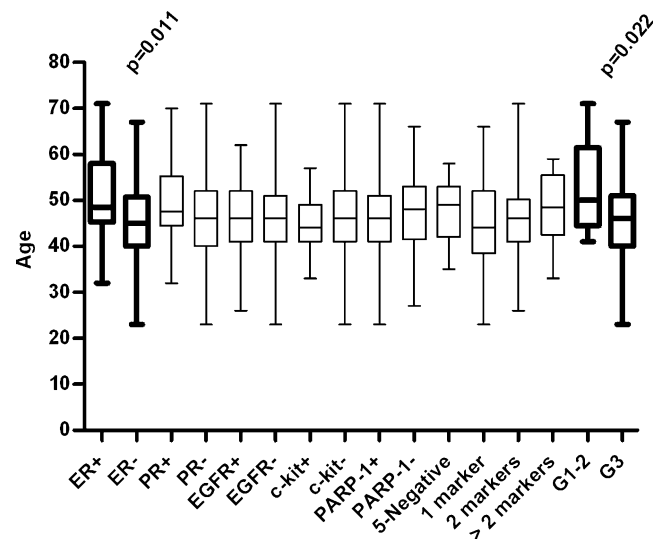
Fig. 3 Differences in age at diagnosis of *BRCA1*-associated breast cancer patients with respect to the expression of predictive markers and tumor grade. The differences of median age between patients with ER+ vs. ER− as well as grades 1–2 vs. 3 are significant

account the fact that immunohistochemical detection of these proteins does not necessarily reflect functional competence, lack of their presence suggests ineffectiveness of a particular targeted therapy, for example, ER—the target of the first targeted therapy in breast cancer—is regarded as one of the most important predictive markers for breast cancers [26]. For ER-positive cancers tamoxifen is an effective treatment regardless of the patient's genotype [27]. Hence, adjuvant anti-estrogen therapy will likely be ineffective in the treatment of breast cancer patients with

Table 4 ER, PR, HER-2, EGFR, c-kit, and PARP-1 immunohistochemical studies in *BRCA1*-associated breast carcinomas reported in the literature and in the current study

References	ER+ % (n+/n)	PR+ % (n+/n)	HER-2+ % (n+/n)	EGFR+ % (n+/n)	c-kit+ % (n+/n)	PARP-1+ % (n+/n)
Johannsson et al. 1997 [32]	8 (3/38)	0 (0/21)	25 (9/36)[a]	–	–	–
Lynch et al. 1998 [33]	23 (5/22)	23 (5/22)	5 (1/22)[a]	–	–	–
Osin et al. 1998 [34]	12 (3/25)	4 (1/25)	–	–	–	–
Eisinger et al. 1999 [35]	11 (3/28)	18 (5/28)	7 (2/28)[a]	–	–	–
Vaziri et al. 2001 [36]	41.4 (12/29)	41.4 (12/29)	9.7 (3/31)[a]	–	–	–
Grushko et al. 2002 [37]	31 (8/26)	–	13 (5/39)	–	–	–
Lakhani et al. 2002, 2005 [5, 6]	10[b]	21[b]	3[b]	67.3[b]	–	–[b]
Quenneville et al. 2002 [38]	15.4 (4/26)[c]	30.8 (8/26)[c]	3.1 (1/32)[a]	–	–	–
Goffin et al. 2003 [39]	23 (7/30)	–	–	–	–	–
Foulkes et al. 2004 [40]	23.6 (49/208)[c]	–	–	–	–	–
Brekelmans et al. 2006 [7]	26.4 (37/140)	30.3 (36/119)	–	–	–	–
Lubiński et al. 2006 [41]	15.5 (18/116)[d]	–	–	–	–	–
Oldenburg et al. 2006 [42]	14.3[e]	21.4[e]	3.9[e]	–	–	–
van der Groep et al. 2006 [43]	–	–	19 (4/21)	67 (14/21)	–	–
Hagen et al. 2007 [44]	20 (8/40)	–	–[f]	–	–	–
Honrado et al. 2007 [45]	28.1 (9/32)	21.2 (7/33)	–	46.4 (13/28)	–	–
Atchley et al. 2008 [46]	30.9 (17/56)	28.9 (15/52)	2.6 (1/38)[g]	–	–	–
Aaltonen et al. 2008 [47]	28.6 (14/49)	20.4 (10/49)	0 (0/35)	–	–	–
Litwiniuk et al. 2008 [48]	14.5 (7/48)	12.5 (6/48)	6.2 (3/48)	–	–	–
Collins et al. 2009 [49]	–	–	–	75 (15/20)[h]	–	–
Evans et al. 2009 [50]	24 (22/87)	–	0 (0/51)	–	–	–
Graeser et al. 2009 [8]	26.9 (158/587)	27 (159/587)	–	–	–	–
Kwong et al. 2009 [51]	48.4 (15/31)[i]	36.7 (11/30)[i]	31.0 (9/29)[i]	–	–	–
Manié et al. 2009 [52]	35 (19/54)	32 (18/57)	0 (0/53)	62 (24/39)	–	–
Joosse et al. 2009 [53]	5.9 (2/34)[a]	5.9 (2/34)[a]	2.9 (1/34)	–	–	–
Lim et al. 2009 [31]	–	–	–	–	48.1 (13/27)	–
Tung et al. 2010 [9]	33.7 (58/172)[j]	34.1 (40/117)[j]	3.4 (4/117)	–	–	–
Domagala et al. (current study)	17.1 (24/140)	13.6 (19/140)	3.6 (5/140)	43.6 (61/140)	14.7 (20/136)	81.9 (113/138)

Only reports based on more than 20 *BRCA1*-associated cancers are included

[a] Different than generally used scoring system

[b] The percentage is based on numbers of scores assigned by two pathologists and not on the number of tumors examined. There were 165 tumors examined for ER/PR and 158 for EGFR

[c] Using both biochemical methods and immunohistochemistry

[d] Early onset breast cancers only

[e] Only percentage given

[f] 15% (six of 40) by FISH

[g] 12% (three of 25) by FISH

[h] Triple-negative *BRCA1*-associated cancers

[i] Includes nine bilateral cancers

[j] 16.3% measured using biochemical methods

ERα deficiency [28]. Targeting HER-2 protein in HER-2-overexpressing breast cancers has resulted in significant clinical benefit for those patients [29]. In a similar manner, patients with *BRCA1*-associated tumors that overexpress EGFR could potentially benefit from EGFR-targeted therapy. However, the relationship of EGFR expression to therapeutic response is not straightforward and has yet to be fully investigated in breast cancer. This is due to various mechanisms of EGFR dysregulation and several compensatory pathways present in tumor cells [30].

We found that 14.7% of *BRCA1*-associated breast carcinomas expressed c-kit. In the only published report, 48.1% of *BRCA1*-associated tumors were c-kit positive [31]. The difference may be explained by a low number of cases (*n*=27), different cut-off level applied to distinguish positive vs. negative cases (authors did not specify it) and different antibody used. Imatinib has proven to be an efficient anti-c-kit-targeted therapy for gastrointestinal stromal tumors; however, little is known about the results of such therapy in breast cancer, and nothing is known about it in *BRCA1*-associated breast cancer, so the clinical value of c-kit therapy in patients with c-kit positive *BRCA1*-associated carcinomas has yet to be established. Our finding that about 15% of *BRCA1*-associated breast cancers express c-kit may help in the proper recruitment of patients for future prospective clinical trials because only those cancers may serve as a target for therapy with imatinib.

In Table 4, the results of our study are compared with major reports in the literature on the immunohistochemical assessment of ER/PR, HER-2, EGFR, and c-kit in *BRCA1*-associated breast carcinomas. The results concerning the expression of steroid receptors and HER-2 confirm previous reports showing that the vast majority of *BRCA1*-associated cancers are steroid receptor and HER-2 negative. However, there are some differences, and they may be attributed to limitations of the previous studies. The majority of published reports (1) were based on a low number of cancers (e.g., only two reports on HER-2 immunohistochemical expression are based on more than 100 cancers [5, 9]); (2) used data concerning ER/PR and HER-2 only from pathology reports; (3) analyzed highly selected cases, i.e., only familial *BRCA1*-associated cancers, or early onset breast cancers, or triple-negative *BRCA1*-associated cancers; (4) applied various criteria for scoring and various immunohistochemical techniques which underwent refinements over the years; and (5) included patients whose cancers were diagnosed before *BRCA1* testing era and this might have selected patients with longer survival, e.g., with ER+ tumors. Our study is the first that is not restricted to familial *BRCA1*-associated cancers but is based on a large group of consecutive *BRCA1*-associated breast cancer patients.

In conclusion, the current treatment recommendations are similar for all patients with *BRCA1*-related cancers. However, we have shown here that these cancers differ with respect to their expression of the proteins that are regarded as targets for specific therapies. Thus, *BRCA1* mutation carriers may have various therapeutic options available. Therefore, we suggest that in order to reveal all therapeutic options accessible to a particular patient, the tumor should be tested for the expression of all five major predictive markers. We believe that in the era of targeted therapy, treatment regimens may become more tumor specific (i.e., more personalized) if the expression of major predictive proteins in tumor cells is taken into account when making treatment decisions about patients with *BRCA1*-associated breast cancers. This data may also be useful when recruiting patients for trials of specific targeted therapy, for example, it seems probable that overall response rate of patients with advanced breast cancer and germline *BRCA1* or *BRCA2* mutations in the phase 2 study of olaparib [22] might have been better if tumors were tested for PARP-1 expression and only patients with PARP-1-positive cancer were enrolled and treated. However, full screening of *BRCA1* may not be readily available in some countries what may hamper recruitment of patients for such trials. Clearly, further prospective studies are needed to reveal the clinical utility of the immunophenotypic predictive profile proposed in this report. Finally, because the majority of *BRCA1*-associated breast cancers are thought to represent the basal-like subtype, such predictive immunophenotype may also be applicable to that subset of breast cancers.

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