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Review

Homologous recombination deficiency in triple negative breast cancer

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ABSTRACT

Triple negative breast cancer (TNBC) represents a heterogeneous subtype of breast cancer characterized by an unfavorable prognosis due to its aggressive biology. The median overall survival (OS) for patients with metastatic TNBC is around 9–12 months with conventional cytotoxic agents. Considering this suboptimal outcome, which is induced despite of medical treatment, new therapeutic strategies would be urgently needed. The ultimate goal of precision medicine is to identify specific molecular alterations that permit considering effective targeted drug(s). Germline BRCA mutations occur in 10–20% of TNBC patients while somatic mutations occur in 3–5% of them. Alterations in the homologous recombination (HR) system are typical of BRCA mutant tumors, but can also be identified in tumors that do not carry this mutation, defining a subgroup of patients referred to as BRCAness. In this review, we focus on the role of homologous recombination deficiency (HRD) as both predictive and prognostic factor in different settings of TNBC patients treated with DNA damaging drugs and poly ADP ribose polymerase (PARP) inhibitors.

Contents

1. Introduction

TNBC is an aggressive tumor accounting for 15% of breast cancers and conventionally defined by the absence of estrogen (ER), progesterone (PR) and human epidermal growth factor 2 (HER2) receptors. TNBC includes a heterogeneous group of tumors, typically occurring in very young patients and which metastasizes commonly by hematogenous spread, having lung and brain as major first sites of metastasis. It usually shows a peak of recurrence during the first three years, with the majority of deaths occurring within the first 5 years [1,2]. Data from The Cancer Genome Atlas (TCGA) and smaller prospective registries identified germline BRCA1/2 deleterious mutations in 10–20% of TNBC patients while somatic mutation in 3–5% of them [3,4].

Tumors with BRCA1/2 mutations typically carry a deficient HR system, cardinal for DNA repair from an insult [5]. DNA is exposed to continuous damage, causing a range of lesions varying from single-strand break (SSB), double-strand break (DSB), bulky

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adducts, base mismatches, insertions, deletions and base alkylation. The type of lesion present on DNA defines the repair mechanism of choice. In presence of DSB, the HR system removes the DSB and uses a homologous DNA template to repair it [6]. This mechanism acts in phase S and G2 of the cell cycle and requires the involvement of proteins encoded by the BRCA1, BRCA2, RAD51 and PALB2 genes. Mutations in one of these key components of DNA repair system are responsible for limited DNA repair capacity [7–11]. Tumors harboring mutations in one of these genes, for example, result more sensible to platinum agents or PARP inhibitors, since these compounds cause DNA inter- and intra-strand crosslinks that can not be recognized and subsequently repaired by HR system, ultimately resulting as defective [12]. Fig. 1 summarizes the key players and their roles in generating the phenotype of HR deficient tumors.

In this review, we focused on the contribution of HRD system as predictive and prognostic factor evaluated in different settings of TNBC patients.

2. BRCA1/2 mutation and homologous recombination (HR) system

Tumors defined as BRCA1/2 deficient show large regions of loss of heterozygosity (LOH), telomeric allelic imbalance (TAI), and large-scale transition (LST) [13]. These three characteristics combined together define the HRD score, which is useful to define the HR deficient status [14–16]. The test used for HRD determination hereon was based on single nucleotide polymorphism (SNP) analysis using two main assays: Myriad genetics and myChoice HRD [17,18]. The first test was analyzed in a retrospective study in TNBC treated with several neoadjuvant regimes [17]. This analysis showed that HRD positive patients were more likely to present a pathological complete response (pCR) than those without this alteration (44% *versus* 8%; $p < 0.01$). The second study analyzed the myChoice assay in 48 TNBC patients treated with carboplatin plus nanoparticle albumin-bound paclitaxel with or without vorinostat [18]. Also here the patients with higher HRD score showed a higher pCR rate than HRD low ones (50% *versus* 7.7%; $p = 0.002$). Although each individual metric is significantly associated with BRCA1/2 status, the combination of the three performed best at distinguishing HRD from non-HRD tumors [13,19]. Alterations in the HR system, while typical of BRCA mutant tumors, can be also identified in tumors not carrying this mutation, defining a subgroup of patients referred to as BRCAness. Telli et al. [20] evaluated in a retrospective trial the combined HRD score, defined as the unweighted numeric sum of LOH, TAI, and LST, and tested the predictive power of a specific HRD threshold. The score was evaluated in a chemonaïve training cohort including 497 TNBC, of

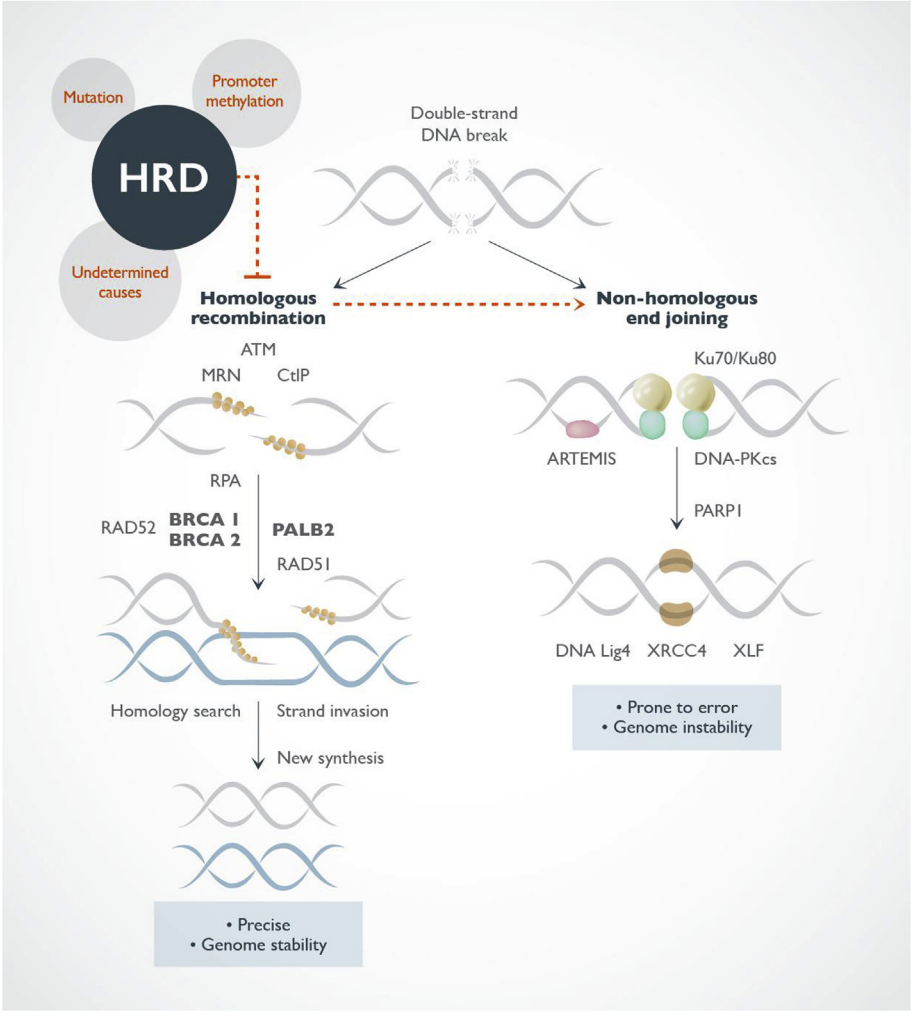

Fig. 1. Representation of how HRD pushes the DNA repair mechanism so as to engage non-homologous pathways, more prone to error and to genome instability.

which 78 had a BRCA1/2 mutation(s); and 561 ovarian tumors, of which 190 were BRCA mutated. BRCA1/2 mutants showed either one deleterious mutation in BRCA1/2 with LOH in the wild-type copy, or two deleterious mutations in the same gene or promoter methylation of BRCA1 with LOH in the wild-type copy. For an optimal accuracy, the threshold for the combined HRD score in the training set of BRCA intact and BRCA deficient subjects was established as ≥42 for both breast and ovarian tumors. HRD status was defined as positive by the presence of either BRCA1/2 mutation or a predefined high HRD score (≥42). HRD was classified as negative if no mutations in BRCA genes were detected or if a low HRD score (<42) was calculated. The validation set included patients enrolled in PrECOG 0105, Cisplatin-1 and Cisplatin-2 studies in which HR score and HRD status were correlated to chemotherapy response [21–23].

The PrECOG 0105 trial was a phase II study that enrolled 93 patients affected by stage I-IIIA TNBC and/or BRCA1/2 germline mutants treated with 4 or 6 cycles of neoadjuvant carboplatin, gemcitabine, and iniparib [21]. The Cisplatin-1 and -2 also analyzed this correlation in the neoadjuvant setting and consist of a 28 and a 51 prospective cohorts of patients, respectively, treated with cisplatin as monotherapy for 4 cycles in the first study and cisplatin plus bevacizumab in the latter [22,23]. In these trials, the HR score - previously calculated in training set - and the HRD status were correlated with response to cisplatin by evaluating the residual cancer burden (RCB) index [24]. Four kinds of response to neoadjuvant treatment were described (RCB 0: pCR, RCB I: minimal residual disease, RCB II: moderate residual disease, and RCB III: extensive residual disease). For each variable, two dichotomous measures for tumor response were used (RCB 0/I: present or not present, and pCR: yes *versus* no). *RCB 0/I yes* includes tumors with pCR or RCB I. *RCB0/I no* corresponds to class RCB II or RCB III. In the PrECOG 0105 study, the HRD score (high/low) was significantly associated with both RCB 0/I ($p = 0.0053$) and pCR ($p = 0.0065$). In the Cisplatin trials, this correspondence was also evidenced by RCB 0/I ($p = 0.0014$) and pCR ($p = 0.0071$). According to the PrECOG 0105, RCB 0/I rates in HRD tumors were 68% *versus* 30% in non-HRD (OR 4.96 CI 1.61–15.3; $p = 0.0036$). pCR rates were 42% in HR deficient tumors *versus* 10% in non-HRD (OR = 6.52, 95% CI, 1.36–31.2; $p = 0.0058$). In the Cisplatin-1 and -2 trials, RCB 0/I rate in HRD tumors were 51.7% *versus* 9.5% in non-HRD ones (OR 10.18, 95% CI 2.00–51.89; $p = 0.0011$). pCR rates were 27.5% in HR deficient and 0% in HR non-deficient tumors (OR 17.00 CI 1.91–2249; $p = 0.0066$). Additionally, in the PrECOG 0105, RCB 0/I rates were higher in BRCA1/2 mutant tumors than wild types (75% *versus* 48%; $p = 0.0037$), also with higher pCR rates (50% *versus* 24%; $p = 0.040$). In the cisplatin trials, probably because the number of mutant tumors was small, BRCA mutation was not significantly associated with RCB 0/I (42.9% *versus* 31.6%; $p = 0.57$) and pCR rates (28.6% vs 13.2%; $p = 0.33$). Rates of RCB 0/I were also evaluated according to the BRCA status in the PrECOG 0105, with 81% *versus* 47% for BRCA1/2 germline mutants when compared to wild types, respectively.

Using the HRD score as a stratifier tool, RCB 0/I rates were 59% in HRD high (≥42) and 32% in HRD low ($p = 0.062$) according to the retrospective analysis from the PrECOG 0105 population; and the pCR was 33% in HRD high *versus* 11% in HRD low tumors ($p = 0.063$). In the Cisplatin trials, a high HRD score was significantly associated with both higher RCB 0/I (52.6% *versus* 10.5% $p = 0.0039$) and pCR rates (26.3% vs 0% $p = 0.018$) when compared to the HRD low subgroup. The multivariate logistic analysis performed on all three above mentioned trials confirmed that the HRD was an independent predictor of RCB 0/I and pCR after adjustment for other clinical covariates.

3. HRD in the adjuvant setting

A study conducted by Sharma et al. evaluated the prognostic role of HRD status, HRD score, and BRCA1 promoter methylation in 425 patients affected by TNBC, comprised either by high risk/node-negative or low-risk/node positive disease and treated with two equivalent dose schedules of doxorubicin and cyclophosphamide [25]. HRD status was positive in 67% of patients, from which 27% had BRCA1/2 mutations. Positive HRD status was associated with a prolonged disease free survival (DFS) (HR 0.72, 95% CI 0.51–1.00; $p = 0.049$) and a non-significant trend toward an improvement in OS (HR 0.71, 95% CI 0.48–1.03; $p = 0.073$) after adjustment for treatment arms and nodal status. The positive HRD status correlation was particularly pronounced during the first 5 years. After this time, the impact on DFS and OS was not significant (HR for DFS 1.21, 95% CI 0.65–2.28; $p = 0.55$ and HR for OS 0.85, 95% CI 0.47–1.53; $p = 0.59$) [20]. Among patients with BRCA1/2 wild type tumors, 40% had an HRD score of ≥42 and it was associated with a higher DFS (HR 0.64, 95% CI 0.43–0.94; $p = 0.023$) as well as OS (HR 0.65, 95% CI 0.47–1.53; $p = 0.59$) when adjusted for treatment and nodal status. Tumor mutational status per se did not impact on DFS ($p = 0.59$) nor OS ($p = 0.9$) after adjustment for treatment and nodal status, probably due to the modest number of patients carrying this alteration. This study underlines the role of HRD positive status in decision making process of patients affected by TNBC, confirming its prognostic role independently from other patient- or tumor-related features. However, no data are available yet to define if this particular category of patients could benefit from specific cytotoxic agents such as taxanes or platinum salts.

4. HRD in the neoadjuvant setting

The phase III neoadjuvant trial BrighTNess randomized clinical stage II-III TNBC to receive a standard neoadjuvant chemotherapy with paclitaxel followed by anthracyclines and cyclophosphamides with or without carboplatin and with or without veliparib [26]. In an analysis conducted on global population independently from BRCA status, veliparib did not provide any benefit in comparison to chemotherapy. pCR, in fact, was significantly higher among patients receiving paclitaxel plus carboplatin plus veliparib than those treated with paclitaxel alone (53% *versus* 31%; $p < 0.0001$) but did not differ between patients receiving paclitaxel plus carboplatin plus veliparib versus paclitaxel plus carboplatin (53% *versus* 58%; $p = 0.36$). In addition, no difference in terms of RCB 0/I were evidenced among these two arms (68% *versus* 70%; $p = 0.739$). The presence of BRCA1/2 mutation was not predictive for pathological response, but the addition of carboplatin increased the rate of pCR (57% in the paclitaxel plus carboplatin plus veliparib group *versus* 50% in the paclitaxel plus carboplatin group *versus* 44% in the paclitaxel alone group) even if this study was not powered to show a significant difference in the attainment of pCR with the addition of carboplatin, with or without veliparib. This study has several limitation such as its retrospective nature, the small sample size and the inclusion of a few number of patients receiving taxanes. However, an important hypothesis arises from this trial that consists of the evaluation of HRD as a new biomarker in predicting the benefit of DNA damaging therapies such as cisplatin, anthracycline, taxane, and cyclophosphamide.

The phase II GeparSixto trial evaluated if the addition of carboplatin to a regimen of paclitaxel, low dose doxorubicin and bevacizumab in TNBC or trastuzumab and lapatinib in HER2 positive disease improved the pCR in stage II-III breast cancer [27]. The results of this trial showed that the addition of carboplatin to a regimen of chemotherapy and targeted therapies significantly increases the proportion of pCR in TNBC (53% *versus* 37%; $p = 0.005$)

but not in HER2 positive disease (32.8% *versus* 36.8%; p = 0.581) [28]. A subgroup analysis of the same study looked at the BRCA1/2 mutations and HRD status as predictive factors for pCR in patients receiving neoadjuvant chemotherapy. HRD, including the unweighted sum of LOH, TAI, and LST and BRCA1/2 mutations, was discovered on 70.5% of TNBC - 60.3% of tumor samples had HRD high without BRCA mutation. HRD high tumors, in its turn, were more likely to achieve a pCR than HRD low ones (55.9% *versus* 29.8%; p = 0.001). In addition, patients treated with carboplatin with a high HRD score showed significantly higher rates of pCR compared to patients treated without a platinum agent (64.9% *versus* 45.2%; p = 0.025).

Another retrospective analysis evaluated the role of HRD status and BRCA1/2 mutations in 45 patients with TNBC and 2 with hormone receptor-positive disease treated with neoadjuvant chemotherapy [29]. Chemotherapy regimens employed included anthracycline (19%), taxanes (9%), and a combination of these two drugs (70%). The mutation on BRCA genes were the following: BRCA1 in 21%, BRCA2 in 6%, BRCA1 and BRCA2 in 2%. The presence of mutations in either or both of genes was not a significant predictor of pCR (OR 2.06, 95% CI 0.52–8.16; p = 0.31) nor of RCB 0/I (OR 1.2, 95% CI 0.34–4.20; p = 0.78). Regarding HRD patients, these had higher probability of achieving pCR after neoadjuvant therapy compared to those that do not present this alteration (OR 13.06, 95% CI 1.52–112.41; p = 0.0028). Also, RCB 0/I was greater in patients with HRD tumors than in non-HRD (OR 5.10, 95% CI 1.42–18.32; p = 0.0087). In addition, HRD score was highly predictive of pCR (p = 0.011) and RCB 0/I (p = 0.0021). Even if this analysis shows several limitations like the small number of enrolled patients, the retrospective nature and the few patients treated with taxanes without anthracycline, it stresses the role of HRD in predicting the response to neoadjuvant chemotherapy in TNBC.

5. HRD in the metastatic and relapsed settings

The phase II BROCADE trial evaluated the combination of veliparib plus temozolomide (VT) versus veliparib plus carboplatin and paclitaxel (VCP) versus placebo plus carboplatin plus paclitaxel (PCP) in metastatic or locally recurred TNBC carrying a BRCA mutation [30]. Median PFS was 7.4 months in VT arm, 14.1 months for VCP and 12.3 months for PCP. Median OS was 19.1 months in the first treatment arm, 28.3 in the second and 25.9 in the third. All these data did not reach statistical significance, and the only noteworthy evidence was an increase in overall response rate (ORR) with the combination of VCP versus PCP (77.8% *versus* 61.3%; p = 0.027). In the VT arm, ORR was 28.6%, a sharp reduction compared with the other two arms of treatment. The small sample size could reasonably be the cause of the trend toward PFS and OS increase on both VCP and PCP arms in BRCA mutated TNBC, hence, no definitive conclusions could be drawn. In addition, the same sample size related issue for metastatic disease before platinum treatment also represents a limit for the sensitivity of the analysis. The ongoing phase III of the current study is investigating the efficacy of veliparib added to carboplatin and paclitaxel versus carboplatin plus paclitaxel in BRCA mutated TNBC (BROCADE 3, NCT02163694).

In the phase III study Treating to New Targets (TNT), patients affected by metastatic TNBC or BRCA1/2 mutation-associated breast cancer were randomized to receive carboplatin versus docetaxel [31]. In overall population the response to carboplatin was not different from docetaxel (31.4% *versus* 35.6%; p = 0.44). However, subjects with a BRCA1/2 germline mutation showed a better response when treated with carboplatin compared to docetaxel (68% *versus* 33%; p = 0.003), with a statistically significant interaction between therapy and BRCA mutation status (p = 0.01). Also, the median PFS was longer in the platinum group compared to the docetaxel group (6.8 *versus* 4.4 months). No difference was found in terms of OS. It is important to consider that 56% of patients received carboplatin at progression. BRCA1 methylation as well as low BRCA1 mRNA levels were not associated to a better response to platinum agent [32]. Those with germline BRCA1/2 mutation or BRCA1 methylation showed a high HRD score, being this last condition not associated with a better response to carboplatin compared with docetaxel (38.2% in the carboplatin arm *versus* 40.4% in the docetaxel arm, a not statistically significant difference [p = 0.75]), differently from what was observed in BRCA mutated tumors. This result was also confirmed when the analysis was limited to high HRD scores, including patients irrespective of their BRCA1/2 mutational status. Also in this case a high score was associated to an ORR of 44.7% with carboplatin *versus* 39.6% with docetaxel and the interaction test evaluating this difference was not statistically significant (p = 0.67). No evidence of increase in median PFS was observed in HRD *versus* non-HRD tumors. These results are similar to those observed in the single-arm, phase II, non randomized TBCRC009 trial in metastatic TNBC, in which patients carrying BRCA1 methylation did not show any response to a platinum agent [33]. The reason could arise from the same condition that the BRCA1 methylation was assessed on primary tissue while the treatment effect was assessed in the metastatic setting. Notwithstanding, this data has a particular value considering that the majority of patients treated in these studies received adjuvant treatment with agents that cause DNA damage, engaging the HR system. Methylation of BRCA1, differently from BRCA mutations, appears to be more revertible. It is postulated that BRCA methylated tumors treated with adjuvant or neoadjuvant chemotherapy actually modify their genetic functionality during treatment since they continue to express the alterations contributing to the HRD score, but drive the tumors towards a soft BRCAness [34,35] phenotype. An example is given by ovarian cancer in which BRCA mutation, but not methylation, was correlated to platinum response, with a biopsy - obtained before and after platinum treatment - showed a reversal of BRCA1 methylation in 31% of tumors [3,36]. The continued presence of methylation was also associated with benefit from a PARP inhibitor [37]. In addition, the Myriad test employed for HRD score definition does not specifically predict response to platinum *versus* docetaxel in advanced disease [20]. In neoadjuvant TNBC, the high HRD score was associated to platinum response but also in this case these studies do not have a comparison arm for testing the interaction between these biomarkers among specific treatment arms. To overcome these caveats, a new whole-genome sequencing analysis methodology (i.e.: HRDetect) allows to evaluate the presence of multiple pathogenomic mutational signatures and to measure the lifetime HRD due to epigenetic transformations. Studies are ongoing to comprehensively validate this tool [19,20,38–42].

6. Discussion

Hereditary BRCA1/2 mutations are found in 5.3% of all breast cancer and it increases to 10–20% in the TNBC subgroup. Somatic mutations were also reported in 3–5% of TNBC [3,4]. BRCA1/2 and PALB2, among many other genes involved in the mechanism of DNA repair, are critical players in the HR repairs of DSB and are associated with TNBC phenotype [43]. Tumors harboring mutation(s) in one of these genes result more sensible to platinum agents or PARP inhibitors since these compounds cause DNA inter and intra-strand crosslinks that can not be recognized and subsequently repaired by the defective HR system [12]. Although BRCA and PALB2 mutations are sensitive indicators of HRD, other combined biomarkers such as LOH, TAI, and LST are useful to better define the HRD status and to

Table 1
Trials looking at the correlation between HRD status and outcomes among different settings of breast cancer populations. *HRD: homologous recombination deficiency; TNBC: triple negative breast cancer; AC: doxorubicin (A) and cyclophosphamide (C); CBDCA: carboplatin; P: paclitaxel; LD: liposomal doxorubicin DFS: disease free survival; OS: overall survival; pCR: pathologic complete response; ns: not significant, T: taxanes; VT: veliparib plus temozolomide; VCP: veliparib + carboplatin + paclitaxel; PCP: placebo + carboplatin + paclitaxel, D: docetaxel; CDDP: cisplatin; ORR: overall response rate.*

Study	Population	Treatment	Study Trials	HRD Status Definition	Primary End-Point	Main Results
SWOG S9313 [25]	Stage II-III TNBC	Combined *vs* sequential adjuvant AC	Retrospective	HRD status[a]	DFS OS	HRD positive status associated with DFS (HR 0.72; p = 0.049). Non significant trend observed with OS (HR 0.71; p = 0.073).
BrighTNess [26]	Stage II-III TNBC	Neoadjuvant P followed by AC ± CBDCA ± veliparib	Prospective	BRCA1/2 deleterious mutation	pCR	BRCA 1/2 mutated tumors *vs* non mutated not predictive for pCR (51% *vs* 48%; p = ns)
GeparSixto [27]	Stage II-III TNBC Stage II-III HER2+	P + LD ± CBDCA plus bevacizumab in TNBC plus trastuzumab + lapatinib in HER2+	Prospective	HRD status[a]	pCR DFS	HRD positive status associated with increased pCR *vs* HRD negative (55.9% *vs* 29.8%; p = 0.001). Adding carboplatin increased pCR in HRD positive but not in HRD negative tumors (64.9% *vs* 45.2%; p = 0.025)
Telli et al. [29]	Stage II-III TNBC Stage II-III HER2+	A, T, or A + T	Retrospective	HRD status[a]	pCR	HRD positive associated with higher pCR rates after neoadjuvant therapy *vs* HRD negative (OR 13.06; p = 0.0028)
BROCADE [44]	Stage III-IV TNBC	VT *vs* VCP *vs* PCP	Prospective	Germline BRCA1/2 mutations	PFS	No statistical significant increase on PFS in BRCA mutated patients *vs* non mutated
TNT [31,32]	Stage III-IV TNBC	CBDCA *vs* D	Prospective	HRD status[a]	ORR	HRD score did not select sensitivity to carboplatin *vs* docetaxel
TBCRC009 [33]	Stage IV TNBC	CBDCA *vs* CDDP	Prospective	BRCA1 promoter methylation	ORR	Presence of BRCA1 promoter methylation did not show particular response to platinum agent

[a] HRD Status was defined positive as either a deleterious tumor BRCA1/2 (tBRCA) mutation or a pre-defined HRD score ≥42. HRD status was defined negative as either an absence of deleterious tumor BRCA1/2 (tBRCA) mutation or a pre-defined HRD score <42.

predict the benefit from platinum agent therapy [13–16]. These alterations, although typical of BRCA mutant tumors, can also be identified in tumors that do not carry this mutation, defining a subgroup of patients referred as BRCAness. A score was identified by a retrospective analysis and defined as the unweighted numeric sum of LOH, TAI, and LST [20]. A score ≥42 or the presence of BRCA1/2 mutations were correlated with objective response to cisplatin by an RCB index. This score was evaluated in different settings of breast cancer and Table 1 outlines the main trials regarding them. From these studies, it clearly emerges that HRD has a potential role as a biomarker to predict which patients could benefit the most from a treatment with DNA damaging drugs such as platinum, PARP inhibitors, anthracyclines and/or cyclophosphamide. Two points remain unclear: the role of HRD in predicting response to combination therapy with cisplatin, anthracyclines, cyclophosphamide and taxanes and the role of platinum with or without PARP inhibitors in tumors with a high HRD score.

Alterations of the DNA repairing system have an important implication taken that tumors with genomic instability are responsible for accumulation of multiple genomic aberration in cancer cells, directly contributing to the mutational load [45]. The consequent neoantigen burden correlates with immunotherapy response and survival outcomes in different solid tumors [46–48]. Mismatch repair (MMR) tumors, for example, are characterized by expression of abundant peptides responsible for elicitation of immune response with a high index of infiltrating lymphocytes in the tumor microenvironment [49–51]. MMR deficient (dMMR) tumors, because of their unstable nature, express high levels of PD-1 and PD-L1 proteins that make this subgroup of tumors more responsible to immunotherapy [51,52]. According to these observations, the FDA accelerated the approval of pembrolizumab for pediatric and adult patients with MMR deficient tumors by early 2017 [53,54]. Still, the relationship between the DNA repair system and immunotherapy response is more complex due to the many DNA lesions types that may elicit different immunological responses. In addition, the DNA repair associated biomarkers were discovered before the next generation sequencing (NGS) era, therefore the employment of this approach could expand the list of features influencing DNA repair system and consequently increase the therapeutic option for patients.

Conflicts of interest

We wish confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our Institutions concerning intellectual property.

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