Evaluation of the methods to identify patients who may benefit from PARP inhibitor use

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Abstract

The effectiveness of poly (ADP-ribose) polymerase inhibitors (PARPi) in treating cancers associated with *BRCA1/2* mutations hinges upon the concept of synthetic lethality and exemplifies the principles of precision medicine. Currently, most clinical trials are recruiting patients based on pathological subtypes or have included *BRCA* mutation analysis (germ line and/or somatic) as part of the selection criteria. Mounting evidence, however, suggests that these drugs may also be efficacious in tumors with defects in other genes involved in the homologous recombination repair pathway. Advances in molecular profiling techniques together with increased research efforts have led to a better understanding of the molecular aberrations underlying this BRCA-like phenotype and helped broaden the concept of BRCAness. Hence, it is likely that the list of predictive biomarkers for PARPi therapy will increase in future. There is currently no gold standard method of testing for PARPi response and no universal guidelines are in place on how to incorporate biomarker testing into routine clinical diagnostics. In this review, we explore the concept of BRCAness and highlight the different methods that have been used to identify patients who may benefit from the use of these anticancer agents. The identification of predictive biomarkers is crucial in improving patient selection and expanding the clinical applications of PARPi therapy.

Introduction

The development of novel therapies targeting specific biological pathways has led to a paradigm shift in the way we approach cancer therapeutics. The use of poly (ADP-ribose) polymerase inhibitors (PARPi), with their selective mechanisms of action involving the DNA damage repair pathways, illustrates this strategy. PARPi are being evaluated in clinical trials either as monotherapeutic agents or in combination with other anticancer therapy to improve therapeutic efficacy. The recruitment of patients for some of these trials has been based on molecular and phenotypic evidence of defects in DNA repair, in particular homologous recombination (HR) repair, such as those with germ line *BRCA* mutations, or included tumor types known to be associated with *BRCA* mutations, such as basal-like or triple-negative breast cancers (TNBC) and high-grade ovarian serous carcinomas (HGOSC). Few studies have included other biomarker analyses as part of their recruitment criteria (Table 1). However, there is increasing evidence to suggest that some patients with tumors less

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Table 1 Clinical trials incorporating molecular biomarkers to select patients for PARP inhibitor therapy. (www.clinicaltrials.gov).

PARP inhibitor	Phase	Other therapeutic agents	Cancer type	Biomarker used for patient stratification	Trial identifier
Talazoparib	1 and 2		Ovarian, primary peritoneal, breast, prostate, pancreas, gastric, other solid tumor	BRCA1/2 mutations	NCT01198546
Talazoparib	2		Ovarian	BRCA1/2 mutations	NCT02326844
Olaparib	2		Ovarian	BRCA1/2 mutations	NCT00494442
Olaparib	2		Breast	BRCA1/2 mutations	NCT00494234
Olaparib	1	Carboplatin	Gynecological and breast	BRCA1/2 mutations; deleterious mutation in DNA repair enzymes	NCT01237067
Talazoparib	3	Physician's choice	Breast	BRCA1/2 mutations	NCT01945775
Veliparib	3	Carboplatin, paclitaxel	Breast	BRCA1/2 mutations	NCT02163694
Olaparib	1	AZD5363	Advanced solid tumors	BRCA1/2 mutations; somatic mutations or other aberrations resulting in hyperactivated PI3K-AKT pathway	NCT02338622
BGB-290	1	Anti-PD-1 monoclonal antibody BGB-A317	Advanced solid tumors	BRCA1/2 mutation; homologous recombination or mismatch repair deficiency	NCT02660034
Olaparib	1	Carboplatin	Breast and ovarian	BRCA1/2 mutations	NCT01445418
Niraparib	3		Ovarian	BRCA1/2 mutations	NCT01847274
Veliparib	2	Cyclophosphamide	Ovarian, fallopian tube, primary peritoneal, breast, non-Hodgkin's lymphoma	BRCA1/2 mutations	NCT01306032
Talazoparib	1		Advanced or recurrent solid tumors	BRCA1/2 mutations	NCT01286987
Olaparib	2	Liposomal Doxorubicin	Ovarian	BRCA1/2 mutations	NCT00628251
Niraparib	1	Temozolomide	Ewing sarcoma	Ewing sarcoma translocation by FISH or RT-PCR	NCT02044120
Olaparib	1		Ovarian	BRCA1/2 mutations	NCT00516373
Olaparib	2	AZD5363, AZD1775, AZD2014	Advanced solid tumors	Mutations in DNA damage repair genes; molecular aberrations of PI3K/AKT pathway; TP53 or KRAS mutations; mutations such as TORC1/2 or TSC1/2 or LKB1 mutations or are PTEN deficient (determined by genetic mutation or by IHC)	NCT02576444
Olaparib	2 and 3	Cediranib maleate, paclitaxel, pegylated liposomal doxorubicin hydrochloride	Ovarian, fallopian tube, primary peritoneal	BRCA1/2 mutations	NCT02502266
Olaparib	3	Cediranib maleate, paclitaxel, carboplatin, gemcitabine hydrochloride	Ovarian, fallopian tube, primary peritoneal	BRCA1/2 mutations	NCT02446600
Olaparib	2	Cediranib maleate	Ovarian, fallopian tube, primary peritoneal	BRCA1/2 mutations	NCT02345265
Olaparib	1 and 2	Cediranib maleate	Ovarian, fallopian tube, primary peritoneal	BRCA1/2 mutations	NCT01116648
Olaparib	1 and 2	AZD2014, AZD5363	Ovarian, fallopian tube, primary peritoneal, endometrial and breast	BRCA1/2 mutations	NCT02208375
Veliparib	3	Carboplatin, paclitaxel	Ovarian, fallopian tube, primary peritoneal	BRCA1/2 mutations	NCT02470585
Olaparib	2		Ovarian and breast	BRCA1/2 mutations	NCT00679783
Talazoparib	2		Advanced cancer	Somatic or germ line BRCA1/2 mutations; genomic alterations in other BRCA pathway genes (ATM, PALB2, Fanconi anemia genes, ARID1A, and other genes, e.g., MER11, RAD50, NBS1, ATR; amplification of EMSY; mutations or homozygous deletions in PTEN and/or PTEN loss by IHC	NCT02286687

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Drug	Phase	Combination	Biomarker	Cancer type	NCT number
Rucaparib	2	Cisplatin	BRCA1/2 mutations	Breast	NCT01074970
Cediranib, Olaparib	1 and 2	MEDI4736	BRCA1/2 mutations	Lung, breast, ovarian, colorectal, prostate	NCT02484404
Olaparib	2	Paclitaxel	ATM IHC	Gastric	NCT01063517
Rucaparib	2		BRCA1/2 mutations	Pancreas	NCT02042378
Talazoparib	2		BRCA1/2 mutations	Breast	NCT02034916
Veliparib	1	FOLFIRI, carboplatin, paclitaxel	BRCA1/2 mutations	Solid tumors	NCT02033551
Olaparib	3	Physician's choice	BRCA1/2 mutations	Breast	NCT02000622
Rucaparib	2		Molecular signature of HR deficiency	Ovarian, fallopian tube, primary peritoneal	NCT01891344
Olaparib	3		BRCA1/2 mutations	Ovarian, fallopian tube, primary peritoneal	NCT01874353
Olaparib	3		BRCA1/2 mutations	Ovarian, fallopian tube, primary peritoneal	NCT01844986
Rucaparib	1 and 2		BRCA1/2 mutations	Ovarian, fallopian tube, primary peritoneal	NCT01482715
Veliparib	1 and 2		BRCA1/2 mutations	Ovarian, fallopian tube, primary peritoneal	NCT01472783
Olaparib	2		BRCA1/2 mutations	Solid tumors	NCT01078662
Niraparib	1 and 2	Bevacizumab	BRCA1/2 mutations, HR deficiency test	Ovarian, fallopian tube, primary peritoneal	NCT02354131
Olaparib	3		BRCA1/2 mutations	Pancreas	NCT02184195
Niraparib	3	Physician's choice	BRCA1/2 mutations	Breast	NCT01905592
Veliparib	2	Temozolomide	BRCA1/2 mutations	Breast	NCT01009788
Veliparib/Dinaciclib	2		BRCA1/2 mutations	Ovarian, fallopian tube, primary peritoneal	NCT01540565
Veliparib	1		BRCA1/2 mutations	Solid tumors	NCT01434316
Veliparib	1	Cyclophosphamide, doxorubicin hydrochloride	BRCA1/2 mutations	Solid tumors and non-Hodgkin's lymphoma	NCT00740805
Veliparib	1	Mitomycin	Deficient for the Fanconi anemia pathway (immunofluorescence assay)	Solid tumors	NCT01017640
Veliparib	2	Gemcitabine hydrochloride, cisplatin	BRCA1/2 mutations	Pancreas	NCT01585805
Veliparib	2 and 3	Temozolomide	MGMT methylation status	Glioblastoma	NCT02152982
Veliparib	1	Carboplatin, fluorothymidine F-18	BRCA1/2 mutations; Deficient for the Fanconi anemia pathway (immunofluorescence assay)	Breast	NCT01251874
Veliparib	2	Carboplatin	BRCA1/2 mutations	Breast	NCT01149083
Veliparib	1		BRCA1/2 mutations	Solid tumors	NCT00892736

commonly associated with *BRCA* mutations, such as melanoma, lung, pancreatic, and prostate cancers, may also respond to PARPi therapy (Fong *et al.* 2009, Paul *et al.* 2011, O'Sullivan *et al.* 2014, Kaufman *et al.* 2015). Furthermore, some tumors in patients without germ line *BRCA* mutations can share similar clinicopathological and molecular characteristics as those occurring in patients with germ line defects, and these could also potentially response to PARPi-based therapy. Some of these tumors have impaired HR repair pathways that may be attributable to a variety of reasons such as *BRCA1* promoter hypermethylation, somatic *BRCA* mutations, or defects in the other genes involved in HR. Therefore, the utility of PARPi in cancer therapeutics is potentially greater than what was initially envisioned.

In this review, we discuss the concept of 'BRCAness' using examples of emerging mechanisms of regulation of HR and evaluate the various methods that can be used to identify patients who may benefit from PARPi therapy.

DNA damage repair pathways and *BRCA*

A variety of endogenous and environmental genotoxic insults can affect the integrity of the human genome. Damaged DNA triggers an activation of the cell cycle checkpoint pathways leading to cell cycle arrest and allows for DNA repair to take place. Single-strand breaks (SSBs) are corrected via the base excision repair (BER), nucleotide excision repair (NER), or mismatch repair pathways (Kinsella 2009), whereas double-strand breaks (DSBs) are repaired by HR, which restores the original nucleotide sequence, or processes such as non-homologous end joining (NHEJ) or single-strand annealing (SSA), which lack fidelity to the germ line DNA sequence (Hoeijmakers 2001, Kinsella 2009) (Fig. 1A). These highly complex and intertwined repair mechanisms are orchestrated by a myriad of enzymes to ensure the integrity of DNA, which is imperative for cell survival.

BRCA1 and *BRCA2* are tumor suppressor genes involved in the repair of DSBs via HR (D'Andrea & Grompe 2003). BRCA1 promotes cell cycle arrest in conjunction with p53 and associates with DNA DSBs. HR repair begins with the degradation of one strand of the DNA at the site of damage, creating a stretch of single-stranded DNA. RAD51 molecules then bind to the single-stranded DNA to form filamentous structures. These RAD51 foci promote recognition of homologous sequences on the sister chromatid and catalyze pairing between the complementary bases in the intact chromosome, ultimately leading to template-dependent DNA synthesis (Wu 2008). BRCA1 and BRCA2 play a role in RAD51 loading, together with other components, such as ATM, H2AX, PALB2, RPA, RAD52, and proteins of the Fanconi anemia pathway (Polo & Jackson 2011). Without functional BRCA1/2, error-prone pathways such as NHEJ are preferentially activated. NHEJ repairs DSBs by ligating the two broken DNA ends without using a homologous DNA sequence to guide repair, often resulting in the introduction of errors at the ligated sites (Davis & Chen 2013) (Fig. 1B). Mutations that are generated can activate oncogenes or inactivate tumor suppressor genes, ultimately leading to carcinogenesis (Tutt & Ashworth 2002, Venkitaraman 2002). In addition to DNA repair, *BRCA1* also contributes to other cellular processes such as transcriptional regulation and chromatin remodeling (Deng 2006, Savage *et al.* 2014).

Given their integral roles in maintaining genomic integrity, patients with germ line mutations of *BRCA1* and *BRCA2* are at an increased risk of developing various malignancies (Venkitaraman 2002). Approximately 5–10% of breast cancers and 1–18% of ovarian cancers occur in patients with germ line *BRCA* mutations (Brody & Biesecker 1998, Pal *et al.* 2005, Walsh *et al.* 2011, Alsop *et al.* 2012). Other less commonly encountered tumors include gastric, pancreatic, prostate, and lung cancers as well as cutaneous melanoma (Brose *et al.* 2002, Leongamornlert *et al.* 2012).
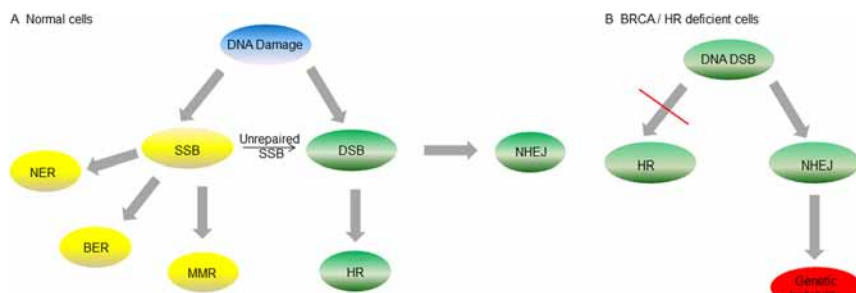
Figure 1
(A) DNA repair pathways in a normal cell. (B) Cells with defects in BRCA or other components of the HR pathway rely on error-prone pathways such as NHEJ for DNA repair, resulting in genetic instability.

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PARP

The PARP proteins are a superfamily of enzymes engaged in a myriad of cellular functions including transcriptional regulation, DNA repair, cell cycle regulation, inflammation, hypoxic response, spindle pole function, oncogene-related signaling, and cell death (Schreiber et al. 2006, Weaver & Yang 2013). A change to the nomenclature has been recently proposed as some members of the group catalyze mono- rather than poly(ADP)ribosylation (Hottiger et al. 2010). PARP1 is the most abundant and best characterized member of the family. It has been implicated in several DNA repair mechanisms such as the repair of SSBs via the BER pathway. It recognizes and binds to sites of SSB, after which it catalyzes the transfer of ADP-ribose molecules from NADC to itself and other acceptor proteins to generate long chains of poly(ADP)ribosylated polymers (Haince et al. 2007). This allows for the recruitment of DNA repair proteins such as DNA polymerase β, DNA ligase III, and scaffolding proteins such as X-ray cross-complementing protein 1 (XRCC1) to sites of SSBs (El-Khamisy et al. 2003, Houtgraaf et al. 2006). PARP1 may also facilitate HR via recruitment of factors such as ATM, Mre11, and Nbs1 to sites of DSBs (Haince et al. 2008) and has been shown to interact with the DNA protein kinase complex involved in NHEJ (Wang et al. 2006) (Fig. 2).

PARP inhibitors and the concept of synthetic lethality

PARPi are a class of drugs that function as catalytic inhibitors that compete with NADC for the substrate-binding site of PARP (Rouleau et al. 2010). They are also postulated to act by 'trapping' PARP at sites of DNA damage, generating a cytotoxic PARP–DNA complex (Murai et al. 2012). Trapped PARP prevents its availability for repair function and secondarily causes replication and transcription fork blockade, resulting in DNA breakage (Helleday 2011). These findings suggest that PARPi have several different modes of action with multiple potential targets in the DNA repair pathway that can result in cancer cell death (Fig. 2).

The early clinical development of PARPi has been focused on targeting cancers associated with BRCA1/2 mutations. This hinges upon the concept of synthetic lethality, whereby a cell harboring one of two gene or protein defects is viable, whereas those containing both defects are not. In this setting, PARP blockade causes replication-associated lesions that cannot be repaired by the defective HR pathway (resulting from BRCA mutations), thereby prompting the activation of compensatory, error-prone DNA repair pathways such as NHEJ that leads to genomic instability, non-viable genetic errors, and, eventually, cell death (Ashworth 2008). This notion was first highlighted in two preclinical studies that
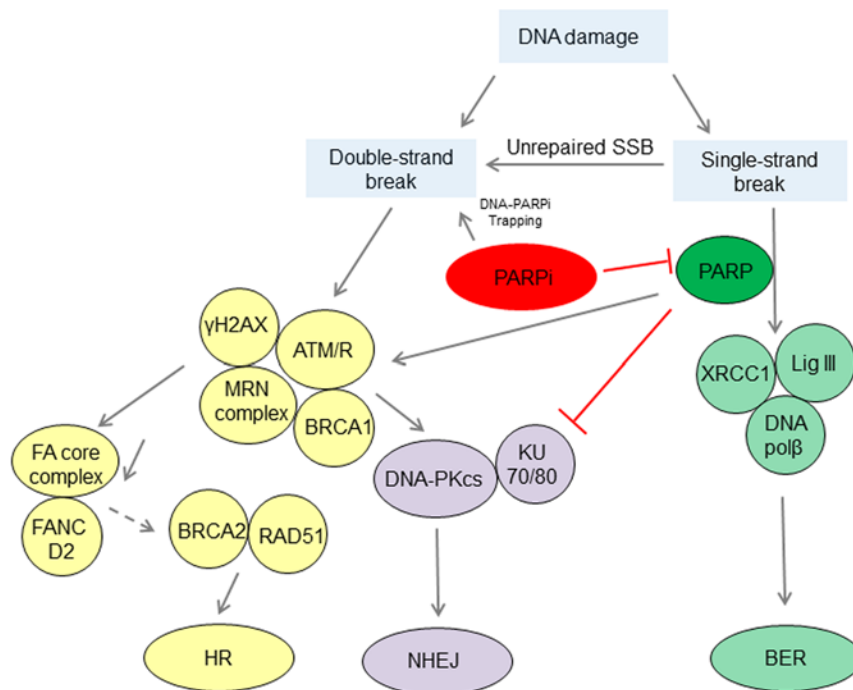
Figure 2
PARP1 binds to DNA single-strand break and catalyzes poly(ADP)ribosylation of itself and acceptor proteins, facilitating the recruitment of DNA repair proteins. In addition to its role in base excision repair, PARP1 plays a role in activating ATM necessary for homologous recombination and inactivating DNA-dependent protein kinase, an important component of non-homologous end-joining. PARP inhibitors have been shown to trap PARP1 on damaged DNA, leading to replication and transcription fork blockage and subsequent double-strand DNA breakage.

established the sensitivity of *BRCA1/2* mutant tumor cells to PARPi (Bryant *et al*. 2005, Farmer *et al*. 2005) and led to subsequent clinical studies in patients with familial *BRCA1/2* mutant breast and ovarian tumors, which provided further clinical evidence for the adoption of this therapeutic approach.

The phase 1 trial evaluating single-agent olaparib (AZD2281) in a cohort of patients enriched for *BRCA1/2* mutation carriers demonstrated clinical benefit in 12/19 (63%) patients with objective responses in 9 (47%) patients (Fong *et al*. 2009). These led to an expansion cohort comprising 50 *BRCA* mutation carriers with ovarian, peritoneal, and fallopian tube cancers, of which 13 were platinum refractory, 24 were resistant to platinum, and 13 were sensitive to platinum. These patients exhibited a response rate (according to RECIST criteria or reduction in serum CA125) of 23%, 45%, and 69%, respectively, and the efficacy of olaparib correlated with *BRCA* mutation and platinum sensitivity (Fong *et al*. 2010). Two subsequent phase 2 trials then established the efficacy of olaparib in familial *BRCA1/2* mutant breast and ovarian cancers, with an overall response rates (ORR) of 41% and 33% noted in the breast and ovarian cohorts respectively (Audeh *et al*. 2010, Tutt *et al*. 2010). In another phase 2 study assessing olaparib as maintenance therapy used in unselected patients with familial or sporadic HGOSC that responded to platinum agents, it was reported that those with germ line or somatic *BRCA1/2* mutations demonstrated the best progression-free survival (PFS). Although a statistically significant improvement in overall survival (OS) was not observed in this cohort, the OS data were insufficiently mature to allow for a properly powered comparison between the treatment groups (Ledermann *et al*. 2012).

In October 2014, the European Medicines Agency (EMA) approved the use of olaparib (Lynparza) as a monotherapy for the maintenance treatment of patients with relapsed, platinum-sensitive epithelial ovarian, fallopian tube, or primary peritoneal cancer with *BRCA1/2* mutations. This was followed 2 months later by an approval by the US Food and Drug Administration (FDA) for the use of Lynparza as a monotherapy for the treatment of patients with deleterious germ line *BRCA*-mutated advanced ovarian cancer who have been treated with three or more prior lines of chemotherapy (Kim *et al*. 2015) based on the findings of a multicenter phase 2 study (Kaufman *et al*. 2015). This approval was granted together with a companion *in vitro* diagnostic assay, BRACAnalysis CDX (Myriad Genetics, Inc, Salt Lake City, UT, USA), which is performed only at the Myriad Genetic Laboratories. The assay allows for the qualitative detection and classification of variants in the protein coding regions and intron/exon boundaries of *BRCA1* and *BRCA2* using genomic DNA derived from whole blood specimens. Single-nucleotide variants and small insertions and deletions (indels) are identified by PCR and Sanger sequencing, whereas large genomic rearrangements such as deletions and duplications are detected using a multiplex PCR assay. Detected variants are classified into one of the five categories: deleterious mutation, suspected deleterious mutation, variant of uncertain significance, favor polymorphism, and polymorphism. As the FDA did not review the hereditary implications of *BRCA* testing in this setting, BRACAnalysis CDx should not be regarded as a surrogate screening test for hereditary cancer (Gunderson & Moore 2015, Kim *et al*. 2015).

BRCAness

Although PARPi have been shown to be effective in patients with germ line *BRCA1/2* mutations, evidence suggests that they may also be of benefit in the treatment of cancers with defects in other components of the DNA damage repair pathways. The concept of 'BRCAness' was introduced to describe the clinical and biological features that some sporadic tumors share with those harboring germ line *BRCA1/2* mutations. This not only includes similar histomorphological features such as basal-like phenotype in breast cancers or high-grade serous morphology in ovarian cancers but also similar immunophenotypic profile (e.g., triple-negative breast cancers), drug sensitivity (e.g., to platinum agents and PARPi), as well as disease prognosis (Turner *et al*. 2004, Tan *et al*. 2008) (Fig. 3). This BRCAness phenotype may be attributed in part to defective HR secondary to several mechanisms, including hypermethylation of the *BRCA1* promoter (Baldwin *et al*. 2000, Esteller *et al*. 2000, Geisler *et al*. 2002, Teodoridis *et al*. 2005), somatic mutations of *BRCA1/2* (Foster *et al*. 1996, Geisler *et al*. 2002, Hilton *et al*. 2002, Hennessy *et al*. 2010, Ledermann *et al*. 2014), *EMSY* amplification (Hughes-Davies *et al*. 2003), or loss-of-function mutations involving other HR pathway genes, including *ATM*, *ATR*, *BARD1*, *BRIP1*, *MRE11A*, *PALB2*, *RAD50*, *RAD51D*, *RAD54*, *NBS1*, *CHEK1*, and *CHEK2*, as well as components of the Fanconi anemia repair pathway (Hughes-Davies *et al*. 2003, Taniguchi *et al*. 2003, Venkitaraman 2003, Dedes *et al*. 2011, Loveday *et al*. 2011, Rigakos & Razis 2012, Strom & Helleday 2012, Lord & Ashworth 2016).
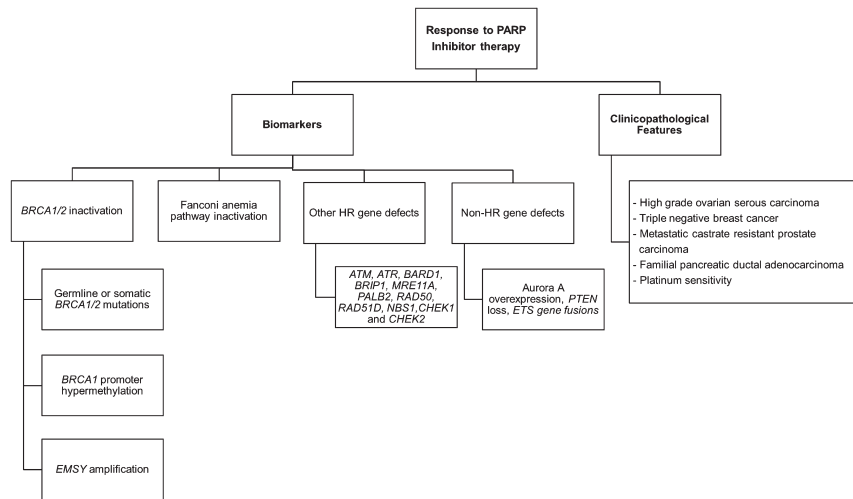
Figure 3
Clinicopathological features and molecular biomarkers associated with response to PARP inhibitor therapy.

Clinical evidence indicates that tumors with this phenotype can respond to PARP inhibition. In a phase 2 trial of olaparib, which included both *BRCA1/2*-mutated and wild-type patients with breast cancer and HGOSC, non-*BRCA* mutant patients who had platinum-sensitive HGOSC showed an ORR of 50% (Gelmon *et al.* 2011). A 17% response rate was also observed in the platinum-resistant cohort without *BRCA1/2* mutation, suggesting an incomplete crossover of platinum sensitivity and PARPi response. Similarly, in a phase 1 study evaluating niraparib in advanced solid tumors enriched for sporadic cancers associated with non-*BRCA* HR repair defects, three patients with partial response and four with stable disease were identified (Schelman *et al.* 2011).

Resistance to PARP inhibition

Conversely, not all patients with mutations in *BRCA1/2* or genes associated with BRCAness will response to PARPi, as different mutations may have differing effects on HR repair function and sensitivities to PARP inhibition. Mouse model studies have shown that a missense mutation in the RING domain of BRCA1, C61G, reduces the ability of BRCA1 to interact with its heterodimerization partner, BRCA1-associated RING domain protein 1 (BARD1), but does not result in PARPi or platinum salt sensitivity (Jaspers *et al.* 2013). The Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA) assessed cancer incidence in more than 31,000 *BRCA1/2* mutation carriers and reported that the risk of developing breast or ovarian cancer is determined by the position and type of mutation, with mutations in different regions of both genes associated with differing risk levels (Rebbeck *et al.* 2015). In comparison, there is very limited understanding of what factors may affect

PARPi responses in the setting of other BRCAness genes. It is also likely that the therapeutic implications may differ in different cancer types, further reinforcing the importance of the context in which *BRCA* and other HR-related genes function in these malignancies.

Differences in treatment response could also result from the development of resistance, and it has been postulated that mechanisms of drug resistance may differ depending on which BRCAness gene is involved (Edwards *et al.* 2008, Sakai *et al.* 2008, Patch *et al.* 2015). Several mechanisms leading to both intrinsic and acquired resistance to PARP inhibitors have been identified. These include secondary mutations that restore the open reading frame and the original function of *BRCA2*, thereby reinstating HR competence (Edwards *et al.* 2008, Sakai *et al.* 2008). Preclinical and clinical evidence indicates that genomic instability promoted by PARPi in HR-deficient cells may result in secondary mutations in the mutated *BRCA1/2* gene with restoration of functional protein expression and induction of PARPi resistance (Edwards *et al.* 2008, Sakai *et al.* 2009, Norquist *et al.* 2011, Barber *et al.* 2013).

Other mechanisms of resistance to PARPi include restoration of HR in *BRCA1* mutant cells via either inactivation of mitotic arrest-deficient 2-like protein 2 (MAD2L2) or P53-binding protein 1 (53BP1) (Bunting *et al.* 2010), both of which are involved in controlling DNA resection at DSBs (Patch *et al.* 2015, Xu *et al.* 2015). *In vitro* and *in vivo* experiments have shown that loss of 53BP1 restores HR and renders *BRCA1*-deficient cells resistant to PARP inhibition (Bouwman *et al.* 2010, Bunting *et al.* 2010). Decreased 53BP1 levels have also been detected in *BRCA1* mutant ovarian carcinoma patients that developed secondary resistance to platinum agents and

PARPi (Johnson *et al*. 2013, Patch *et al*. 2015). Thus, 53BP1 expression may be of use in predicting response to PARPi.

Biomarker testing

Despite a greater understanding of the molecular aberrations associated with a BRCA-like phenotype, the identification of patients who will respond to therapy still presents considerable challenges. This is due to the lack of a unifying morphological phenotype, the varied components of the repair pathways, and numerous potential mechanisms of drug resistance. The development of predictive biomarkers and diagnostic assays that will allow for robust patient selection remains an important area of research. At present, there is no gold standard method to reliably identify such patients for PARPi therapy, although various biomarkers have been explored, including testing for *BRCA* mutations (both germ line and somatic) or genetic defects in the other genes involved in HR.

Germ line *BRCA* mutations

BRCA1/2 germ line variant screening has traditionally been performed using a combination of Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA). Sanger sequencing is able to detect small variants such as the deletion or insertion of single bases, whereas MLPA identifies large gene rearrangements, such as the deletion or duplication of one or more exons. However, these methods are time consuming and costly (Ruiz *et al*. 2014) and they generally require a significant input of good-quality DNA. The adoption of next-generation sequencing (NGS) has allowed for massive parallel sequencing of multiple genes, ranging from multi-gene panels to whole exomes and genomes (Feliubadalo *et al*. 2013, Hernan *et al*. 2012). In comparison to direct Sanger sequencing, NGS allows for faster, more efficient, high-throughput testing at a considerably lower cost (Harismendy *et al*. 2009). With NGS, results can consistently be provided within a clinically useful time frame, allowing their incorporation into treatment decisions. However, these sequencing techniques have limited ability to detect structural gene rearrangements and may need to be supplemented by other methods such as MLPA to ensure that the full spectrum of genetic aberrations are accounted for (Patch *et al*. 2015). Furthermore, most NGS platforms require substantial bioinformatic input for the analysis and interpretation of sequencing data, and this has been a considerable hurdle for laboratories considering switching

to NGS. Wider availability of more affordable testing may also result in a greater volume of tests performed, placing an increased need for better integration between oncology and clinical genetic services (George 2015).

BRCA inactivation in sporadic cancers

Another important mechanism of BRCAness is the presence of somatic mutations in *BRCA1/2*, which has been identified in some sporadic ovarian and breast cancers. Hennessy and colleagues performed *BRCA1/2* sequencing using snap-frozen tumor tissue from 235 unselected ovarian cancers and identified mutations in 19% (31 *BRCA1* and 13 *BRCA2* mutations) of tumors with the vast majority occurring in high-grade serous carcinomas. In 28 samples, where germ line DNA was also available, 42.9% of the *BRCA1* mutations and 28.6% of the *BRCA2* mutations were found to be purely somatic. *BRCA1/2* mutations were associated with improved PFS after platinum-based chemotherapy in univariate and multivariate analyses. BRCA1/2 deficiency, defined as *BRCA1/2* mutations or expression loss, was present in 30% of tumors and was also significantly associated with PFS (Hennessy *et al*. 2010). Using targeted capture and massively parallel genomic sequencing, Pennington *et al*. identified germ line (24%) and somatic (9%) mutations in one or more of 13 HR genes, including B*RCA1*, *BRCA2*, *ATM*, *BARD1*, *BRIP1*, *CHEK1*, *CHEK2*, *FAM175A*, *MRE11A*, *NBN*, *PALB2*, *RAD51C*, and *RAD51D*, in patients with ovarian, fallopian tube, and peritoneal carcinomas. Interestingly, both serous and non-serous carcinomas were found to have comparable HR mutation rates. The presence of germ line and somatic HR mutations was highly predictive of primary platinum sensitivity ($P = 0.0002$) and improved overall survival ($P = 0.0006$), with a median overall survival of 66 and 59 months in cases with germ line or somatic HR mutations, respectively, compared with 41 months for those with no HR mutations (Pennington *et al*. 2014). Similarly, the Cancer Genome Atlas (TCGA) reported that up to 6.3% of HGOSC harbor somatic mutations in *BRCA1/2* (Cancer Genome Atlas Research Network 2011).

Somatic *BRCA1/2* mutations have also been identified in breast cancers albeit in smaller numbers. In the TCGA cohort, approximately 20% of TNBCs had either germ line ($N = 12$) or somatic ($N = 8$) *BRCA1/2* mutations, whereas in another study, one of 77 TNBC was found to harbor a somatic *BRCA* mutation (Gonzalez-Angulo *et al*. 2011). These findings highlight the need to evaluate tumors

for somatic disruption of the *BRCA* pathway in patients lacking germ line *BRCA* mutations.

There are, however, several challenges to be met in trying to detect somatic *BRCA* mutations in tumor samples. First, some specimens may be small (e.g., biopsies), with limited DNA yield that may be of poor quality, thus constraining the analysis that can be undertaken. This poses a significant challenge with respect to accurate detection, characterization, and interpretation of sequence variants in *BRCA1/2*. Second, tumor samples are also histologically heterogeneous, and DNA derived from tumor tissue will invariably contain admixed DNA from normal cells. Consequently, methods for somatic mutation detection have to be able to detect DNA changes that may be present in a low proportion of the total DNA isolated from the sample. Traditional Sanger sequencing techniques for BRCA testing generally require good-quality, high-molecular-weight input DNA of high yield, usually extracted from blood, and may not be suitable for analysis of formalin-fixed paraffin-embedded (FFPE) tumor tissue, where the extracted DNA is typically of poorer quality, more fragmented, and of low yield. In addition, Sanger sequencing may not be sensitive enough to detect low-level somatic changes present in tumor tissue. In comparison, NGS methods require less input DNA, as the reactions can be highly multiplexed and have the potential to detect variants at low allele frequency (Sims *et al.* 2014); thus, the use of NGS may offer a potential solution to this challenging type of analysis (Ellison *et al.* 2015). Lastly, formalin fixation can lead to deamination and cross-linkage of DNA, resulting in sequencing artifacts. This issue may be resolved by duplicate analysis starting from the original DNA, as artifacts will generally be randomly distributed and should not be present in both samples.

Epigenetic mechanisms of gene inactivation may occur as an alternative to genetic mutation in the silencing of *BRCA* (Jones & Baylin 2002). Aberrant methylation of cytosine residues in CpG dinucleotides in the promoter region results in transcriptional silencing of the gene. Aberrant methylation of the *BRCA1* promoter is identified in up to 14% of sporadic breast cancers (Catteau *et al.* 1999, Esteller *et al.* 2000, Rice *et al.* 2000) and 31% of ovarian cancers (Catteau *et al.* 1999, Baldwin *et al.* 2000, Esteller *et al.* 2000, Geisler *et al.* 2002). *BRCA1* methylation is associated with decreased BRCA1 transcript in breast cancer (Esteller *et al.* 2000, Rice *et al.* 2000) and with decreased/absent protein expression by immunohistochemical analysis in breast (Matros *et al.* 2005) and ovarian cancers (Baldwin *et al.*

2000). Evidence to support the etiological importance of *BRCA1* methylation is derived from the similarities in morphological, immunohistochemical, and molecular phenotypes between these tumors and familial *BRCA1* cancers. For example, *BRCA1*-methylated breast cancers have a higher histological grade, are more likely to be ER negative, lack *ERBB2* amplification, and frequently show amplification of *c-MYC*, similar to familial *BRCA1* cancers (Catteau *et al.* 1999, Esteller *et al.* 2000, Grushko *et al.* 2004). Data from expression microarrays also suggest that the expression profile of sporadic tumors with *BRCA1* methylation is similar to those with germ line *BRCA1* mutation (Sorlie *et al.* 2003). Thus, patients with hypermethylated *BRCA1* may benefit from PARPi therapy, although it is possible that they may not demonstrate the same degree of drug sensitivity as patients with germ line *BRCA* mutations (Cancer Genome Atlas Research Network 2011). This was alluded to in a study by Ruscito and colleagues, who showed that even though 14.8% of HGOSC had hypermethylation in a selected region of the *BRCA1* promoter, this had no effect on the PFS or OS rate in patients treated with conventional chemotherapy (Ruscito *et al.* 2014). Methylation status of the *BRCA* genes may be investigated using a variety of methods including direct bisulfite sequencing, methylation-specific PCR, methylation microarrays, pyrosequencing, and NGS, the choice of which would be dependent on factors such as the type of biological samples and tumor content present (Ibragimova & Cairns 2011).

In contrast to *BRCA1*, the *BRCA2* promoter is rarely hypermethylated; however, transcriptional down-regulation of *BRCA2* is frequently associated with amplification of the *EMSY* gene, which has been reported in up to 13% of sporadic breast cancers and 17% of high-grade sporadic ovarian cancers (Hughes-Davies *et al.* 2003).

BRCA immunohistochemistry

Absent immunohistochemical (IHC) expression of BRCA1 can be attributed to a variety of mechanisms including germ line or somatic mutation and promoter hypermethylation and therefore may have utility as a surrogate marker for *BRCA1* loss. A study evaluating IHC expression and mutational status of *BRCA1* in HGOSC reported that IHC was an accurate and highly reproducible method for detecting germ line, somatic, or epigenetic mechanisms of *BRCA1* loss. These findings were consistent with results from other studies examining the use of BRCA1 IHC (Byrne *et al.* 2000, Vaz *et al.* 2007, Garg *et al.* 2013).

Endocrine-Related Cancer

Lesnock *et al.* also reported that decreased BRCA1 expression was associated with a 36-month survival improvement in patients with ovarian carcinoma treated with cisplatin-based intraperitoneal chemotherapy and thus may be a useful biomarker for selecting patients for this form of therapy (Lesnock *et al.* 2013).

The other advantages of using IHC is that it is a relatively simple and cost-efficient technique that is performed in most pathology laboratories and can be easily repeated, thus allowing its use as a dynamic biomarker throughout the disease course as methylation status changes or secondary gain-of-function mutations accumulate. These findings support the incorporation of BRCA1 IHC testing for patient selection in clinical trials, particularly in the setting of recurrent disease (Meisel *et al.* 2014).

Detecting BRCAness

Relying solely on *BRCA* mutations to drive PARP-directed therapeutics will undoubtedly exclude a significant proportion of patients with defects in other HR genes who may also benefit from PARPi therapy. The major challenge lies in the development and validation of robust assays to identify or even quantify HR deficiencies in tumor samples so as to improve patient selection for PARPi therapy (Do & Chen 2013).

The development of new molecular profiling techniques has allowed for more rapid and in-depth characterization of the frequency of HR gene mutations in different cancer types (Cancer Genome Atlas Research Network 2011, Patch *et al.* 2015). Data from the TCGA study that profiled 489 HGOSC using a combination of whole exome sequencing, mRNA, microRNA, methylome, and DNA copy number profiling identified alterations in at least one HR-modulating gene in about 50% of tumors. These included germ line *BRCA1* (9%) or *BRCA2* (8%) mutations, somatic mutations of *BRCA1/2* (3%), amplification of *EMSY* (13%), *PTEN* mutations (7%), hypermethylation of the *RAD51* homolog *RAD51C* (3%), mutations in *ATM* or *ATR* (2%), and *FANC* mutations (5%) (Cancer Genome Atlas Research Network 2011). In another study, whole-genome profiling of 114 tumor samples from 92 patients with HGOSC was performed and again, either germ line or somatic defects in BRCAness-associated genes, were identified in approximately half of the samples analyzed, including mutations involving *BRCA1* (promoter hypermethylation was also observed), *BRCA2*, *PTEN*, *RAD51B*, *BRIP1* (also known

as *FANCJ*), *CHEK2*, *FANCI*, and *RAD51C* (Patch *et al.* 2015). Using targeted capture and massively parallel genomic sequencing, Pennington *et al.* assessed 390 ovarian carcinomas for germ line and somatic loss-of-function mutations in 30 genes, including *BRCA1/2*, and 11 other genes in the HR pathway (Pennington *et al.* 2014). Almost 1/3 of these tumors were found to have deleterious germ line (24%) and/or somatic (9%) mutations in at least one of the 13 HR genes (*BRCA1*, *BRCA2*, *ATM*, *BARD1*, *BRIP1*, *CHEK1*, *CHEK2*, *FAM175A*, *MRE11A*, *NBN*, *PALB2*, *RAD51C*, and *RAD51*), and the presence of these mutations was predictive of primary platinum sensitivity and improved OS (Pennington *et al.* 2014).

Other malignancies such as breast, prostate, and pancreatic cancers can also exhibit defects in HR-modulating genes. Data from the TCGA analysis of 507 breast cancer patients revealed the presence of germ line mutations in *BRCA1*, *BRCA2*, *ATM*, *BRIP1*, *CHEK2*, *NBS1*, *PTEN*, or *RAD51C* in 47 patients and 20% of TNBC had either germ line or somatic *BRCA1/2* mutations (Cancer Genome Atlas Research Network 2012). Using panel sequencing, Beltran *et al.* reported that 12% and 8% castration-resistant prostate cancer (CRPC) harbor *BRCA2* and *ATM* mutations (Beltran *et al.* 2013), respectively, whereas whole exome and transcriptome profiling of 150 metastatic CRPC revealed the presence of at least one mutation in a BRCAness-associated gene in more than 19% of tumors (Robinson *et al.* 2015). Data from a phase 2 clinical trial assessing the efficacy of olaparib in the treatment of metastatic CRPC showed that 88% of patients with homozygous deletions and/or deleterious mutations in a HR repair gene responded to olaparib (Mateo *et al.* 2015). Similarly, whole-genome and whole-exome sequencing of 100 pancreatic ductal adenocarcinoma samples demonstrated that 24% possessed either a germ line or a somatic mutation in *BRCA1*, *BRCA2*, or *PALB2*, whereas 8% had *ATM* mutations (Biankin *et al.* 2012).

Results from these studies support the use of massively parallel sequencing analysis in prospectively designed trials for the selection of patients likely to respond to PARP inhibition. The heterogeneous genetic profile of these tumors make them ideal candidates for panel testing, where comprehensive analysis of multiple genes are performed in parallel. This approach shows clear advantages over sequential testing of multiple genes, which is costly, requires a larger input of DNA, and cannot be realistically performed in a clinically relevant time frame. However, one of the

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major difficulties encountered when using panel testing is the reporting of genetic mutations for which no clear evidence of a causal link exist (Pennington *et al*. 2014). Determining which of the mutations identified are actually pathogenic and how other variants should be reported will be issues that have to be considered when adopting these sequencing techniques.

It can be further argued that targeted genotyping assays, even when using custom panels, may prove to be inadequate, considering the extensive repertoire of genes involved in DNA repair response and the heterogeneous nature of their genetic/epigenetic inactivation. Therefore, array-based strategies, such as gene expression arrays, have been used to identify gene expression patterns characteristic of defects in the HR pathway, whereas comparative genomic hybridization (CGH) arrays have been utilized to identify patterns characteristic of the genomic instability inherent in BRCAness, as well as to identify specific genomic changes selected for in these tumors.

Gene expression signatures

Using previously published gene expression data from familial and sporadic ovarian cancers, a group of investigators identified a *BRCA*-like 60-gene signature profile, which was initially validated in ten tumor biopsies from six patients with germ line *BRCA1/2* mutations and then in 70 patients with sporadic ovarian cancers, where it was shown to correlate with responsiveness to platinum agents and PARPi. In addition, this 'BRCAness' profile correlated with the ability to form RAD51 foci in *BRCA2*-mutated pancreatic cancer cell line Capan-1 and was also able to predict for PARPi sensitivity, suggesting that the profile may be detecting a pattern of gene expression that more globally reflects the status of HR, independent of cell lineage (Konstantinopoulos *et al*. 2010).

The use of similar gene expression profiling approaches has also allowed for the classification of breast cancers. Familial *BRCA1* mutant tumors were identified to segregate strongly with basal-type sporadic cancers (Sorlie *et al*. 2003), indicating that basal-type sporadic tumors and familial *BRCA1* mutant tumors could have similar etiologies. Larsen and colleagues analyzed 55 familial *BRCA1/2* mutant and 128 sporadic breast tumors to derive a transcriptional signature that was able to predict *BRCA* mutant cancers in an independent data set (Larsen *et al*. 2013). Other investigators have also used transcriptional profiles

derived from cell lines with either known HR gene defects (Daemen *et al*. 2012, Peng *et al*. 2014) or PARPi sensitivity to generate BRCAness signatures.

Mutational signatures

Impaired DNA repair results in genomic alterations in tumors and contributes to genomic scars that may allow tumors to be molecularly stratified. Evidence suggests that HR-deficient tumors have a unique mutation signature that results from the use of error-prone DSB repair mechanisms (Tutt *et al*. 2001, Xia *et al*. 2001). Stratton and colleagues performed a meta-analysis using sequence data from more than 7000 cancers and identified 20 distinct, conserved mutation signatures across a wide variety of tumor types. A signature, characterized by the presence of relatively small deletions (up to 50 bp), was strongly associated with *BRCA1/2* mutations in breast, ovarian, and pancreatic cancers. Interestingly, a subset of tumors lacking *BRCA1/2* mutations also exhibited this signature, suggesting the presence of other DNA repair defects in these neoplasms (Alexandrov *et al*. 2013). Indeed, many of these mutation signatures associated with *BRCA* mutant tumors, which are often defined by the frequency of specific types of structural rearrangement, can also be found in sporadic tumors. Importantly, these signatures may correlate with response to PARPi therapy (Watkins *et al*. 2014) and therefore could be used to identify tumors with a BRCAness phenotype. Various high-throughput genomic profiling techniques including array CGH and single-nucleotide polymorphism (SNP) profiling have been used to identify these structural rearrangements.

Array CGH is a technique that assesses DNA copy number changes, such as amplification and deletion, by hybridizing labeled tumor DNA with differently labeled normal control DNA to metaphase chromosomes. Genomic profiling of breast cancers by aCGH identified a *BRCA* profile that was 91% accurate in distinguishing *BRCA*-mutated cancers from sporadic, non-hereditary cancers (Joosse *et al*. 2009). In this study, two of 48 hereditary but non-*BRCA*-mutated cancers were found to be *BRCA*-like based on 191 discriminatory features. One of these cases had methylation of *BRCA1*. These investigators then developed a *BRCA2* classifier using the same technique, and this showed 89% sensitivity and 84% specificity when applied to the validation cohort. A similar approach was used to stratify patients with breast cancer into four distinct subgroups: simple-profile, BRCA1-related, BRCA2-related, and genomic instability-high group 3 (GII-high-III) (Stefansson *et al*. 2009).

The *BRCA1/2*-related cancers showed several large regions of genomic gains or deletions, which was also noted in sporadic cancers within the GII-high-III group, suggesting that these sporadic breast cancers may also harbor defects in HR that could render them sensitive to PARPi therapy. aCGH has also been used to reveal patterns of copy number changes in *BRCA1/2* mutant breast tumors that was predictive of a favorable response to high-dose carboplatin-based chemotherapy in sporadically occurring TNBC or ER+/HER2- tumors (Lips *et al.* 2011*b*, Vollebergh *et al.* 2011, 2014).

Lips *et al.* further utilized their aCGH-based classifier of *BRCA1*-mutated breast cancers to establish a MLPA assay, capable of identifying patients with both *BRCA1*-mutated tumors and sporadic cancers with a *BRCA1*-like genomic profile, which had significantly better recurrence-free survival when treated with alkylating chemotherapy (Lips *et al.* 2011*a*). MLPA is a method based on amplification and relative quantification of the ligated adjacent probes, which can target up to 50 different genomic regions that show diagnostically or clinically significant copy number changes in patient samples. It is a rapid, cost-efficient method that requires only a small amount of input DNA that can easily be obtained from FFPE specimens and therefore may be more suitable than aCGH for routine clinical application. As the MLPA assay can identify *BRCA1*-deficient breast cancer patients, this method could be applied both for clinical genetic testing and as a predictor of sensitivity to agents such as PARPi. In the clinical genetic setting, the classifier could be used in addition to conventional *BRCA1* mutation testing, as a tool to classify *BRCA1* variants of unknown significance or to identify potential *BRCA1* mutations other than the mutations that are currently screened for (Lips *et al.* 2011*a*). However, compared with aCGH, the assay only interrogates a limited number of genomic loci, thus potentially limiting its use as a discovery platform.

SNP analysis has been used to develop allelic imbalance assays that are associated with a BRCAness profile (Abkevich *et al.* 2012, Birkbak *et al.* 2012, Wang *et al.* 2012, Timms *et al.* 2014, Marquard *et al.* 2015). Tutt *et al.* profiled 126 TNBC using the genome-wide Affymetrix SNP 6.0 array and demonstrated that allelic imbalanced copy number aberrations (AiCNA) were more prevalent in tumors that responded to platinum agents (Watkins *et al.* 2015). Timms and colleagues from Myriad Genetics Inc. used SNP profiling to develop a HR deficiency (HRD) assay, which combines three different DNA-based metrics of genomic instability: loss of heterozygosity, telomeric allelic imbalance, and large-scale state transitions, from which a HRD score could then be calculated. The HRD score was reported to be predictive of response to platinum-based chemotherapy and/or PARPi in patients with TNBC or BRCA1/2 mutation-associated breast cancer and high-grade ovarian carcinomas, and also identified responders lacking a deleterious *BRCA1/2* mutation (Timms *et al.* 2014, Brown 2015, Wilcoxen KM 2015, Telli *et al.* 2016). The assay can be performed using DNA extracted from FFPE tumor tissue and thus can be translated into a clinical setting.

The utility of these mutation signatures is also being evaluated in clinical trials. ARIEL2 (NCT# 01891344) is a phase 2 study evaluating the use of rucaparib for the treatment of women with relapsed, high-grade serous or endometrioid ovarian, fallopian tube, or primary peritoneal cancer. One of the aims of this study is to define a molecular signature of HR defect in these tumors that correlates with response to rucaparib, by quantifying the extent of loss of heterozygosity (LOH) across the tumor genome (McNeish 2015).

In addition to assessing for structural rearrangement signatures, the mutational burden (total number of exome mutations) of a tumor may also be predictive of a BRCAness phenotype. Two studies of ovarian cancers showed that tumors with high mutational burdens were more responsive to platinum-based chemotherapy, suggesting that this could have some utility as a BRCAness biomarker (Birkbak *et al.* 2013, Lord & Ashworth 2016).

The major drawbacks of most of these techniques used to derive mutational signatures, which will limit their role in routine clinical application, includes (1) the requirement for fresh/frozen tumor tissue for analysis, which may not always be available, (2) cost (hardware and manpower), and (3) a need for substantial bioinformatic support to analysis and interpret the data, which may not be available in most diagnostic laboratories.

Functional biomarkers

An alternative approach to identifying BRCAness would be to develop functional assays that can detect HR defects regardless of the type of genetic aberrations that are present.

One of the specific cellular hallmarks of HR is the localization of RAD51 to defined foci in the nucleus after DNA damage and this can be readily identified by immunofluorescent microscopy. Cells deficient in BRCA1/2 or other HR factors do not form RAD51 nuclear foci efficiently following DNA damage, suggesting

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that this could be a functional biomarker of HR dysfunction (Michels *et al.* 2014, Oplustilova *et al.* 2012). Immunofluorescence-based detection of RAD51 foci, coupled with quantification of additional DNA repair-related proteins, has been successfully applied to classify tumors as either HR-competent (RAD51 foci-positive) or HR-defective (RAD51 foci-negative), with a strong predictive value for chemotherapy response (Willers *et al.* 2009, Graeser *et al.* 2010, Mukhopadhyay *et al.* 2010). Mukhopadhyay *et al.* investigated RAD51 foci formation in 25 primary ovarian cancer cultures (Mukhopadhyay *et al.* 2010). Failure to form foci correlated with *ex vivo* sensitivity to rucaparib with a negative predictive value of 100% and positive predictive value of 93%. The authors also reported a 50–60% incidence of HR deficiency in sporadic ovarian cancers, which further reinforces the need for biomarkers of HR dysfunction instead of relying only on *BRCA* mutation status. Studies of breast cancers and AML also demonstrated that DNA damage-induced RAD51 foci can be detected in different tumor types. This approach has been applied to FFPE samples of breast cancer biopsied after neoadjuvant anthracycline therapy, where it has been shown that a low RAD51 score correlated with high histological grade, high proliferative index, and a TNBC phenotype and was predictive of complete pathological response to chemotherapy (Graeser *et al.* 2010). There are, however, limitations in the use of RAD51 foci as a biomarker for HR proficiency. First, RAD51 foci cannot be detected at baseline and must be induced by DNA damage such as that caused by ionizing radiation or PARPi. Second, the expression of RAD51 is restricted to the S and G2 phases of proliferating cells and cannot be detected in tumor cells that are dormant or arrested in the G1 phase (Graeser *et al.* 2010).

Another key protein involved in DNA repair is histone H2AX, which also assembles as foci at DNA DSBs in HR competent cells, where it becomes phosphorylated to form gH2AX and creates a focus for the accumulation of DNA repair and chromatin remodeling proteins. Detection of this phosphorylation event by immunofluorescence using an antibody to gH2AX has been explored as a marker to assess the extent of DNA damage in patients (Redon *et al.* 2010). The use of a combination of gH2AX/RAD51 immunofluorescence was investigated in primary ovarian cancer cell cultures (Mukhopadhyay *et al.* 2010) and primary AML cultures (Gaymes *et al.* 2009), where it has been demonstrated that raised gH2AX and decreased RAD51 foci expression predict for PARPi sensitivity. These techniques have been applied to both fresh and FFPE tissues and are also currently being

validated in circulating tumor cells in the peripheral blood of patients as a marker to gauge response to PARPi therapy (Yap *et al.* 2011).

Preclinical studies of *BRCA2*-mutated cancer cell lines show that PARP hyperactivation is associated with sensitivity to PARPi (Gottipati *et al.* 2010). A surrogate for PARP activity is the detection of PAR polymers (Turner & Ashworth 2011). High PAR levels, as assessed by western blotting or immunohistochemistry, have been shown to predict sensitivity of human cancer cells to PARPi *in vitro* and *in vivo* and may be used to predict sensitivity to PARPi (Ji *et al.* 2011, Michels *et al.* 2013).

Other biomarkers

Other non-HR-related proteins whose function can impact HR may also contribute to 'BRCAness'. The mitotic serine/threonine kinase Aurora A is frequently amplified in cancer. In preclinical models, overexpression of Aurora A impairs RAD51 recruitment, thus disabling DSB repair and sensitizing cells to PARP inhibition (Michels *et al.* 2014).

Aberrations in the PI3 kinase/AKT/mTOR pathways, such as *PTEN* loss or activating *PI3KCA* mutations, may also correlate with PARPi sensitivity. *PI3K* inhibition has been reported to decrease the expression of *BRCA1* and *BRCA2*, thereby disabling HR-mediated repair and sensitizing *BRCA* wild-type TNBC cells and xenografts to PARP inhibition (Ibrahim *et al.* 2012). *PTEN* is a tumor suppressor gene that inactivates the *PI3K/AKT* pathway. Loss of *PTEN* function through mutations, deletions, or promoter hypermethylation occurs in several cancer types. Loss of *PTEN* has been postulated to result in HR dysfunction (Mendes-Pereira *et al.* 2009). Shen *et al.* reported that *PTEN* depletion impairs HR-driven repair by decreasing the expression of RAD51 (Shen *et al.* 2007). Increased PARPi sensitivity was demonstrated in cell line studies and xenograft models with *PTEN* mutation (Mendes-Pereira *et al.* 2009). There is also clinical evidence that PARPi may have a therapeutic utility in *PTEN*-deficient endometrial cancer (Dedes *et al.* 2010, Forster *et al.* 2011). However, results from a study on prostate cancers failed to show any significant correlation between *PTEN* status and sensitivity to PARPi or with the expression of genes associated with HR (Fraser *et al.* 2012).

ETS gene fusions are present in several cancer types, including Ewing's sarcoma and prostate cancer. ETS is a transcription factor with a high number of *BRCA1/2* binding motifs and may repress the *BRCA* promoter upon its activation by the mitogen-activated protein

kinase pathway (Sharrocks 2001, Baker *et al*. 2003). Gene fusion between the *ERG* (a member of the *ETS* family) proto-oncogene and *TMPRSS2* promoter is observed in approximately 50% of prostate cancers and results in aberrant androgen-dependent ERG expression (Tan *et al*. 2014) and promotes carcinogenesis (Tomlins *et al*. 2005). Preclinical studies have shown that PARP1 directly interacts with ERG to inhibit *ETS* gene fusion protein activity. In turn, inhibition of PARP1 reduces *ETS*-positive, but not *ETS*-negative, prostate cancer xenograft growth (Brenner *et al*. 2011). PARPi have also demonstrated anti-tumor activity in the treatment of Ewing's sarcoma in xenograft and cell lines studies (Brenner *et al*. 2012). More studies will be needed to validate the clinical predictive value of *ETS* gene fusions.

Future directions

The advent of new molecular profiling techniques has led to a greater understanding of cancer biology. It is increasingly evident that aside from the traditional clinicopathological classification of tumors, their unique molecular characteristics do allow them to be further stratified according to their genetic profile. This has been illustrated by the various studies that have been performed to identify tumors, other than those with *BRCA1/2* mutations, which may response to PARP inhibition. However, many questions remain to be addressed. First, it is still uncertain what constitutes the best predictor of response to this group of drugs. Most clinical trials are recruiting patients based on pathological subtypes, e.g., TNBC and HGOSC, or have included *BRCA* mutation analysis (germ line and/or somatic) as part of the selection criteria. Few have incorporated other biomarker testing such as other HR gene defects, as part of their enrolment requirements. However, there are clinical trials that have included biomarker analysis as part of their outcome measures and it is hopeful that results from these studies may help shed more light as to which additional biomarkers should be incorporated for future trials. Thus at present, although it appears that *BRCA* remains the top candidate biomarker that should be tested to assess for PARPi response, it is highly plausible that this list will be expanded in future. Second, there is also no clear evidence as to what drives PARPi response in tumors without a mutation in a canonical HR repair gene. As PARP proteins have mechanisms of action beyond DNA repair, the benefits of PARPi are likely not going to be just confined to *BRCA* or even BRCAness-associated tumors. Knowledge of other mechanistic properties of

PARPi will also influence the choice of combination therapy in different cancer types. Lastly, a more in-depth understanding of the mechanisms of drug resistance is needed, as it is uncertain whether these mechanisms may differ depending on which BRCAness gene is involved or whether cross-resistance to other DNA damaging agents can occur. This again will affect the choice of combination therapy and the sequence in which these drugs should be administered.

PARPi have been yielding promising results in several clinical trials. They have been employed both as monotherapy and in combination therapy with radiation, chemotherapeutic agents, as well as other molecularly targeted agents, for several different cancer types, and have demonstrated a relatively good safety profile. The identification and validation of predictive biomarkers of response to PARPi is an important area of ongoing research, which will lead to wider clinical applications for these drugs. However, the incorporation of biomarker testing into routine clinical diagnostics also presents significant challenges. Undoubtedly, there will be more practical requirements for assays used in the clinical setting than those used for research purposes only. These tests should be readily reproducible, feasible using standard equipment, cost-effective, and can be completed in an appropriate time frame for them to be clinically relevant. It will also be an advantage if the test can be performed using FFPE tissue, as this is the material routinely available in pathology laboratories. Some centers have incorporated *BRCA* testing (germ line and/or somatic) as part of their clinical workflow, using methods discussed previously. However, as more biomarkers are added to the list, single gene testing will undoubtedly prove to be less attractive compared with multiplex assays such as panel testing that allows for multiple genes to be interrogated simultaneously in a clinically relevant time frame. We envision that future testing strategies will adopt the latter approach to allow for more efficient and cost-effective testing. Importantly, this approach will also allow for the incorporation of predictors of drug resistance, which will help refine the selection of patients likely to respond to these therapeutic agents.

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