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

Diana Lim[1] and Joanne Ngeow[2,3]

[1]Department of Pathology, National University Health System, Singapore, Singapore
[2]Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore, Singapore
[3]Cancer Genetics Service, Division of Medical Oncology, National Cancer Centre, Singapore, Singapore

Correspondence should be addressed to J Ngeow
Email
Joanne.Ngeow.Yuen.Yie@singhealth.com.sg

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.

Key Words

- PARP inhibitors
- BRCA
- BRCAness
- homologous recombination
- biomarkers

Endocrine-Related Cancer (2016) **23**, R267–R285

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

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0116
© 2016 Society for Endocrinology
Printed in Great Britain
Published by Bioscientifica Ltd.

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	NCT01989546
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

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0116
© 2016 Society for Endocrinology
Printed in Great Britain
Published by Bioscientifica Ltd.

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

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0116
© 2016 Society for Endocrinology
Printed in Great Britain
Published by Bioscientifica Ltd.

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).

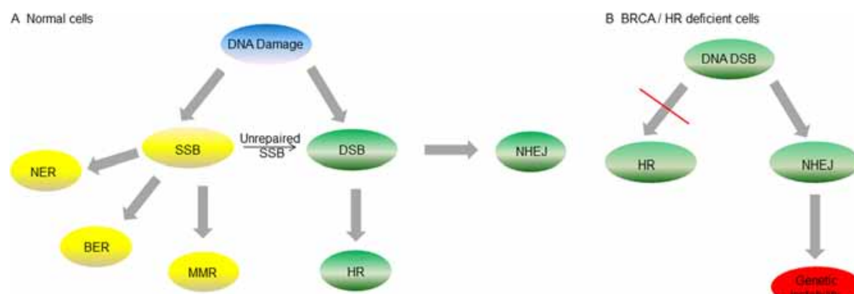

Figure 1
(A) DNA repair pathways in a normal cell. (B) Cells with defects in BRCA or other components of the HR pathway rely on error-prone pathways such as NHEJ for DNA repair, resulting in genetic instability.

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0116
© 2016 Society for Endocrinology
Printed in Great Britain
Published by Bioscientifica Ltd.

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

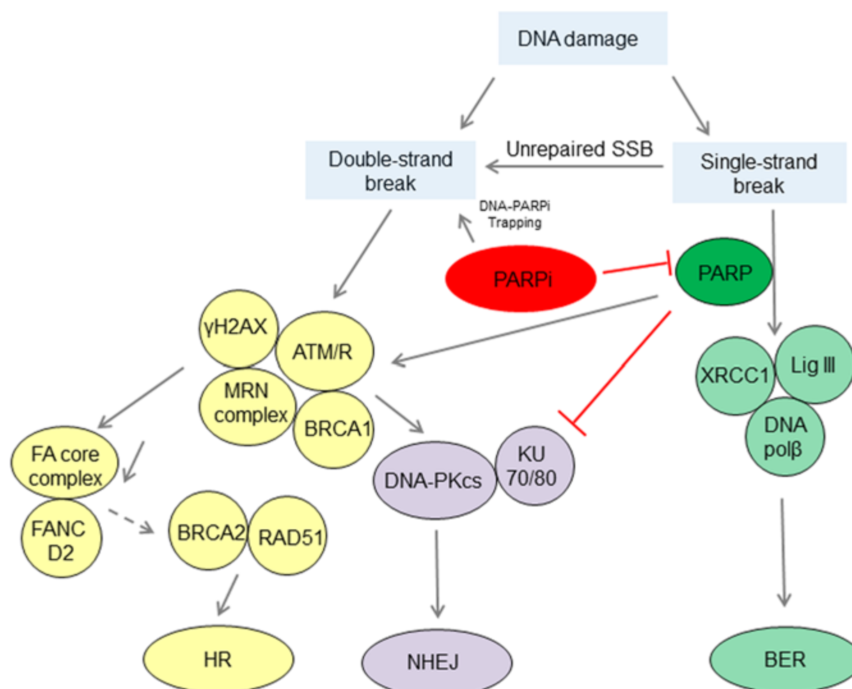

Figure 2
PARP1 binds to DNA single-strand break and catalyzes poly(ADP)ribosylation of itself and acceptor proteins, facilitating the recruitment of DNA repair proteins. In addition to its role in base excision repair, PARP1 plays a role in activating ATM necessary for homologous recombination and inactivating 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.

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0116
© 2016 Society for Endocrinology
Printed in Great Britain
Published by Bioscientifica Ltd.

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).

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0116
© 2016 Society for Endocrinology
Printed in Great Britain
Published by Bioscientifica Ltd.

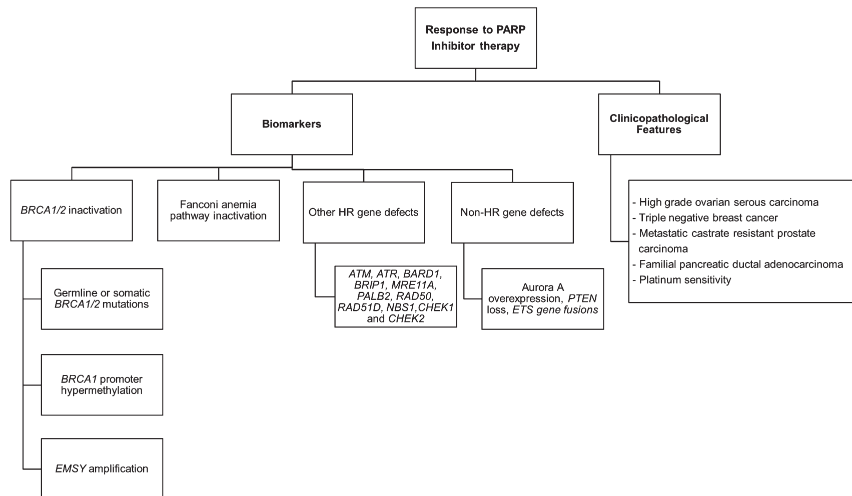

Figure 3
Clinicopathological features and molecular biomarkers associated with response to PARP inhibitor therapy.

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

DOI: 10.1530/ERC-16-0116
© 2016 Society for Endocrinology
Printed in Great Britain

Published by Bioscientifica Ltd.

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 *BRCA1*, *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

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0116
© 2016 Society for Endocrinology
Printed in Great Britain
Published by Bioscientifica Ltd.

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).

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0116
© 2016 Society for Endocrinology
Printed in Great Britain
Published by Bioscientifica Ltd.

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

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0116
© 2016 Society for Endocrinology
Printed in Great Britain
Published by Bioscientifica Ltd.

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).

Endocrine-Related Cancer

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0116
© 2016 Society for Endocrinology
Printed in Great Britain
Published by Bioscientifica Ltd.

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

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0116
© 2016 Society for Endocrinology
Printed in Great Britain
Published by Bioscientifica Ltd.

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

© 2016 Society for Endocrinology
Printed in Great Britain

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.

Declaration of interest
No author had any financial or personal relationships that could inappropriately influence or bias this work.

Funding
This research did not receive any specific grant from any funding agency in the public, commercial, or not-for-profit sector.

© 2016 Society for Endocrinology
Printed in Great Britain

Acknowledgments
J N is a recipient of the National Medical Research Council (NMRC) Transition Award.

References

Abkevich V, Timms KM, Hennessy BT, Potter J, Carey MS, Meyer LA, Smith-McCune K, Broaddus R, Lu KH, Chen J, *et al.* 2012 Patterns of genomic loss of heterozygosity predict homologous recombination repair defects in epithelial ovarian cancer. *British Journal of Cancer* **107** 1776–1782. (doi:10.1038/bjc.2012.451)

Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, Bignell GR, Bolli N, Borg A, Borresen-Dale AL, *et al.* 2013 Signatures of mutational processes in human cancer. *Nature* **500** 415–421. (doi:10.1038/nature12477)

Alsop K, Fereday S, Meldrum C, deFazio A, Emmanuel C, George J, Dobrovic A, Birrer MJ, Webb PM, Stewart C, *et al.* 2012 BRCA mutation frequency and patterns of treatment response in BRCA mutation-positive women with ovarian cancer: a report from the Australian Ovarian Cancer Study Group. *Journal of Clinical Oncology* **30** 2654–2663. (doi:10.1200/JCO.2011.39.8545)

Ashworth A 2008 A synthetic lethal therapeutic approach: poly(ADP) ribose polymerase inhibitors for the treatment of cancers deficient in DNA double-strand break repair. *Journal of Clinical Oncology* **26** 3785–3790. (doi:10.1200/JCO.2008.16.0812)

Audeh MW, Carmichael J, Penson RT, Friedlander M, Powell B, Bell-McGuinn KM, Scott C, Weitzel JN, Oaknin A, Loman N, *et al.* 2010 Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer: a proof-of-concept trial. *Lancet* **376** 245–251. (doi:10.1016/S0140-6736(10)60893-8)

Baker KM, Wei G, Schaffner AE & Ostrowski MC 2003 Ets-2 and components of mammalian SWI/SNF form a repressor complex that negatively regulates the BRCA1 promoter. *Journal of Biological Chemistry* **278** 17876–17884. (doi:10.1074/jbc.M209480200)

Baldwin RL, Nemeth E, Tran H, Shvartsman H, Cass I, Narod S & Karlan BY 2000 BRCA1 promoter region hypermethylation in ovarian carcinoma: a population-based study. *Cancer Research* **60** 5329–5333.

Barber LJ, Sandhu S, Chen L, Campbell J, Kozarewa I, Fenwick K, Assiotis I, Rodrigues DN, Reis Filho JS, Moreno V, *et al.* 2013 Secondary mutations in BRCA2 associated with clinical resistance to a PARP inhibitor. *Journal of Pathology* **229** 422–429. (doi:10.1002/path.4140)

Beltran H, Yelensky R, Frampton GM, Park K, Downing SR, MacDonald TY, Jarosz M, Lipson D, Tagawa ST, Nanus DM, *et al.* 2013 Targeted next-generation sequencing of advanced prostate cancer identifies potential therapeutic targets and disease heterogeneity. *European Urology* **63** 920–926. (doi:10.1016/j.eururo.2012.08.053)

Biankin AV, Waddell N, Kassahn KS, Gingras MC, Muthuswamy LB, Johns AL, Miller DK, Wilson PJ, Patch AM, Wu J, *et al.* 2012 Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes. *Nature* **491** 399–405. (doi:10.1038/nature11547)

Birkbak NJ, Wang ZC, Kim JY, Eklund AC, Li Q, Tian R, Bowman-Colin C, Li Y, Greene-Colozzi A, Iglehart JD, *et al.* 2012 Telomeric allelic imbalance indicates defective DNA repair and sensitivity to DNA-damaging agents. *Cancer Discovery* **2** 366–375. (doi:10.1158/2159-8290.CD-11-0206)

Birkbak NJ, Kochupurakkal B, Izarzugaza JM, Eklund AC, Li Y, Liu J, Szallasi Z, Matulonis UA, Richardson AL, Iglehart JD, *et al.* 2013 Tumor mutation burden forecasts outcome in ovarian cancer with BRCA1 or BRCA2 mutations. *PLoS ONE* **8** e80023. (doi:10.1371/journal.pone.0080023)

Bouwman P, Aly A, Escandell JM, Pieterse M, Bartkova J, van der Gulden H, Hiddingh S, Thanasoula M, Kulkarni A, Yang Q, *et al.* 2010 53BP1 loss rescues BRCA1 deficiency and is associated with triple-negative and BRCA-mutated breast cancers. *Nature Structural & Molecular Biology* **17** 688–695. (doi:10.1038/nsmb.1831)

Brenner JC, Ateeq B, Li Y, Yocum AK, Cao Q, Asangani IA, Patel S, Wang X, Liang H, Yu J, *et al.* 2011 Mechanistic rationale for inhibition of poly(ADP-ribose) polymerase in ETS gene fusion-positive prostate cancer. *Cancer Cell* **19** 664–678. (doi:10.1016/j.ccr.2011.04.010)

Brenner JC, Feng FY, Han S, Patel S, Goyal SV, Bou-Maroun LM, Liu M, Lonigro R, Prensner JR, Tomlins SA, *et al.* 2012 PARP-1 inhibition as a targeted strategy to treat Ewing's sarcoma. *Cancer Research* **72** 1608–1613. (doi:10.1158/0008-5472.CAN-11-3648)

Brody LC & Biesecker BB 1998 Breast cancer susceptibility genes. BRCA1 and BRCA2. *Medicine* **77** 208–226. (doi:10.1097/00005792-199805000-00006)

Brose MS, Rebbeck TR, Calzone KA, Stopfer JE, Nathanson KL & Weber BL 2002 Cancer risk estimates for BRCA1 mutation carriers identified in a risk evaluation program. *Journal of the National Cancer Institute* **94** 1365–1372. (doi:10.1093/jnci/94.18.1365)

Brown R, Timms K, Paul J, Hughes E, El-Bahrawy M, Steel JH, Kalva S, Liu X, Wang Y, Rama NR, *et al.* 2015 Homologous recombination (HR) deficiency, tumor BRCA1/2 mutations (tmBRCA) and association with response and outcome following platinum monotherapy in high grade serous ovarian cancer (HGSOC). *Journal of Clinical Oncology* **33** 5576.

Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, Kyle S, Meuth M, Curtin NJ & Helleday T 2005 Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature* **434** 913–917. (doi:10.1038/nature03443)

Bunting SF, Callen E, Wong N, Chen HT, Polato F, Gunn A, Bothmer A, Feldhahn N, Fernandez-Capetillo O, Cao L, *et al.* 2010 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. *Cell* **141** 243–254. (doi:10.1016/j.cell.2010.03.012)

Byrne TJ, Reece MT, Adams LA, Lane MA & Cohn GM 2000 An antibody assay predictive of BRCA1 mutations in ovarian tumors and normal tissue. *Oncology Reports* **7** 949–953.

Cancer Genome Atlas Research Network 2011 Integrated genomic analyses of ovarian carcinoma. *Nature* **474** 609–615. (doi:10.1038/nature10166)

Cancer Genome Atlas Research Network 2012 Comprehensive molecular portraits of human breast tumours. *Nature* **490** 61–70. (doi:10.1038/nature11412)

Catteau A, Harris WH, Xu CF & Solomon E 1999 Methylation of the BRCA1 promoter region in sporadic breast and ovarian cancer: correlation with disease characteristics. *Oncogene* **18** 1957–1965. (doi:10.1038/sj.onc.1202509)

D'Andrea AD & Grompe M 2003 The Fanconi anaemia/BRCA pathway. *Nature Reviews. Cancer* **3** 23–34. (doi:10.1038/nrc970)

Daemen A, Wolf DM, Korkola JE, Griffith OL, Frankum JR, Brough R, Jakkula LR, Wang NJ, Natrajan R, Reis-Filho JS, *et al.* 2012 Cross-platform pathway-based analysis identifies markers of response to the PARP inhibitor olaparib. *Breast Cancer Research and Treatment* **135** 505–517. (doi:10.1007/s10549-012-2188-0)

Davis AJ & Chen DJ 2013 DNA double strand break repair via non-homologous end-joining. *Translational Cancer Research* **2** 130–143. (doi:10.3978/j.issn.2218-676X.2013.04.02)

Dedes KJ, Wetterskog D, Mendes-Pereira AM, Natrajan R, Lambros MB, Geyer FC, Vatcheva R, Savage K, Mackay A, Lord CJ, *et al.* 2010 PTEN deficiency in endometrioid endometrial adenocarcinomas predicts sensitivity to PARP inhibitors. *Science Translational Medicine* **2** 53ra75. (doi:10.1126/scitranslmed.3001538)

Dedes KJ, Wilkerson PM, Wetterskog D, Weigelt B, Ashworth A & Reis-Filho JS 2011 Synthetic lethality of PARP inhibition in cancers lacking BRCA1 and BRCA2 mutations. *Cell Cycle* **10** 1192–1199. (doi:10.4161/cc.10.8.15273)

© 2016 Society for Endocrinology
Printed in Great Britain

Deng CX 2006 BRCA1: cell cycle checkpoint, genetic instability, DNA damage response and cancer evolution. *Nucleic Acids Research* **34** 1416–1426. (doi:10.1093/nar/gkl010)

Do K & Chen AP 2013 Molecular pathways: targeting PARP in cancer treatment. *Clinical Cancer Research* **19** 977–984. (doi:10.1158/1078-0432.CCR-12-0163)

Edwards SL, Brough R, Lord CJ, Natrajan R, Vatcheva R, Levine DA, Boyd J, Reis-Filho JS & Ashworth A 2008 Resistance to therapy caused by intragenic deletion in BRCA2. *Nature* **451** 1111–1115. (doi:10.1038/nature06548)

El-Khamisy SF, Masutani M, Suzuki H & Caldecott KW 2003 A requirement for PARP-1 for the assembly or stability of XRCC1 nuclear foci at sites of oxidative DNA damage. *Nucleic Acids Research* **31** 5526–5533. (doi:10.1093/nar/gkg761)

Ellison G, Huang S, Carr H, Wallace A, Ahdesmaki M, Bhaskar S & Mills J 2015 A reliable method for the detection of BRCA1 and BRCA2 mutations in fixed tumour tissue utilising multiplex PCR-based targeted next generation sequencing. *BMC Clinical Pathology* **15** 5. (doi:10.1186/s12907-015-0004-6)

Esteller M, Silva JM, Dominguez G, Bonilla F, Matias-Guiu X, Lerma E, Bussaglia E, Prat J, Harkes IC, Repasky EA, *et al.* 2000 Promoter hypermethylation and BRCA1 inactivation in sporadic breast and ovarian tumors. *Journal of the National Cancer Institute* **92** 564–569. (doi:10.1093/jnci/92.7.564)

Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, Santarosa M, Dillon KJ, Hickson I, Knights C, *et al.* 2005 Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* **434** 917–921. (doi:10.1038/nature03445)

Feliubadalo L, Lopez-Doriga A, Castellsague E, del Valle J, Menendez M, Tornero E, Montes E, Cuesta R, Gomez C, Campos O, *et al.* 2013 Next-generation sequencing meets genetic diagnostics: development of a comprehensive workflow for the analysis of BRCA1 and BRCA2 genes. *European Journal of Human Genetics* **21** 864–870. (doi:10.1038/ejhg.2012.270)

Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M, Mortimer P, Swaisland H, Lau A, O'Connor MJ, *et al.* 2009 Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. *New England Journal of Medicine* **361** 123–134. (doi:10.1056/NEJMoa0900212)

Fong PC, Yap TA, Boss DS, Carden CP, Mergui-Roelvink M, Gourley C, De Greve J, Lubinski J, Shanley S, Messiou C, *et al.* 2010 Poly(ADP)-ribose polymerase inhibition: frequent durable responses in BRCA carrier ovarian cancer correlating with platinum-free interval. *Journal of Clinical Oncology* **28** 2512–2519. (doi:10.1200/JCO.2009.26.9589)

Foster KA, Harrington P, Kerr J, Russell P, DiCioccio RA, Scott IV, Jacobs I, Chenevix-Trench G, Ponder BA & Gayther SA 1996 Somatic and germline mutations of the BRCA2 gene in sporadic ovarian cancer. *Cancer Research* **56** 3622–3625.

Forster MD, Dedes KJ, Sandhu S, Frentzas S, Kristeleit R, Ashworth A, Poole CJ, Weigelt B, Kaye SB & Molife LR 2011 Treatment with olaparib in a patient with PTEN-deficient endometrioid endometrial cancer. *Nature Reviews. Clinical Oncology* **8** 302–306. (doi:10.1038/nrclinonc.2011.42)

Fraser M, Zhao H, Luoto KR, Lundin C, Coackley C, Chan N, Joshua AM, Bismar TA, Evans A, Helleday T, *et al.* 2012 PTEN deletion in prostate cancer cells does not associate with loss of RAD51 function: implications for radiotherapy and chemotherapy. *Clinical Cancer Research* **18** 1015–1027. (doi:10.1158/1078-0432.CCR-11-2189)

Garg K, Levine DA, Olvera N, Dao F, Bisogna M, Secord AA, Berchuck A, Cerami E, Schultz N & Soslow RA 2013 BRCA1 immunohistochemistry in a molecularly characterized cohort of ovarian high-grade serous carcinomas. *American Journal of Surgical Pathology* **37** 138–146. (doi:10.1097/PAS.0b013e31826cabbd)

Gaymes TJ, Shall S, MacPherson LJ, Twine NA, Lea NC, Farzaneh F & Mufti GJ 2009 Inhibitors of poly ADP-ribose polymerase (PARP) induce apoptosis of myeloid leukemic cells: potential for therapy of myeloid leukemia and myelodysplastic syndromes. *Haematologica* **94** 638–646. (doi:10.3324/haematol.2008.001933)

Geisler JP, Hatterman-Zogg MA, Rathe JA & Buller RE 2002 Frequency of BRCA1 dysfunction in ovarian cancer. *Journal of the National Cancer Institute* **94** 61–67. (doi:10.1093/jnci/94.1.61)

Gelmon KA, Tischkowitz M, Mackay H, Swenerton K, Robidoux A, Tonkin K, Hirte H, Huntsman D, Clemons M, Gilks B, *et al.* 2011 Olaparib in patients with recurrent high-grade serous or poorly differentiated ovarian carcinoma or triple-negative breast cancer: a phase 2, multicentre, open-label, non-randomised study. *Lancet Oncology* **12** 852–861. (doi:10.1016/S1470-2045(11)70214-5)

George A 2015 UK BRCA mutation testing in patients with ovarian cancer. *British Journal of Cancer* **113** Suppl 1 S17–S21. (doi:10.1038/bjc.2015.396)

Gonzalez-Angulo AM, Timms KM, Liu S, Chen H, Litton JK, Potter J, Lanchbury JS, Stemke-Hale K, Hennessy BT, Arun BK, *et al.* 2011 Incidence and outcome of BRCA mutations in unselected patients with triple receptor-negative breast cancer. *Clinical Cancer Research* **17** 1082–1089. (doi:10.1158/1078-0432.CCR-10-2560)

Gottipati P, Vischioni B, Schultz N, Solomons J, Bryant HE, Djureinovic T, Issaeva N, Sleeth K, Sharma RA & Helleday T 2010 Poly(ADP-ribose) polymerase is hyperactivated in homologous recombination-defective cells. *Cancer Research* **70** 5389–5398. (doi:10.1158/0008-5472.CAN-09-4716)

Graeser M, McCarthy A, Lord CJ, Savage K, Hills M, Salter J, Orr N, Parton M, Smith IE, Reis-Filho JS, *et al.* 2010 A marker of homologous recombination predicts pathologic complete response to neoadjuvant chemotherapy in primary breast cancer. *Clinical Cancer Research* **16** 6159–6168. (doi:10.1158/1078-0432.CCR-10-1027)

Grushko TA, Dignam JJ, Das S, Blackwood AM, Perou CM, Ridderstrale KK, Anderson KN, Wei MJ, Adams AJ, Hagos FG, *et al.* 2004 MYC is amplified in BRCA1-associated breast cancers. *Clinical Cancer Resarch* **10** 499–507. (doi:10.1158/1078-0432.CCR-0976-03)

Gunderson CC & Moore KN 2015 BRACAnalysis CDx as a companion diagnostic tool for Lynparza. *Expert Review of Molecular Diagnostics* **15** 1111–1116. (doi:10.1586/14737159.2015.1078238)

Haince JF, Kozlov S, Dawson VL, Dawson TM, Hendzel MJ, Lavin MF & Poirier GG 2007 Ataxia telangiectasia mutated (ATM) signaling network is modulated by a novel poly(ADP-ribose)-dependent pathway in the early response to DNA-damaging agents. *Journal of Biological Chemistry* **282** 16441–16453. (doi:10.1074/jbc.M608406200)

Haince JF, McDonald D, Rodrigue A, Dery U, Masson JY, Hendzel MJ & Poirier GG 2008 PARP1-dependent kinetics of recruitment of MRE11 and NBS1 proteins to multiple DNA damage sites. *Journal of Biological Chemistry* **283** 1197–1208. (doi:10.1074/jbc.M706734200)

Harismendy O, Ng PC, Strausberg RL, Wang X, Stockwell TB, Beeson KY, Schork NJ, Murray SS, Topol EJ, Levy S, *et al.* 2009 Evaluation of next generation sequencing platforms for population targeted sequencing studies. *Genome Biology* **10** R32. (doi:10.1186/gb-2009-10-3-r32)

Helleday T 2011 The underlying mechanism for the PARP and BRCA synthetic lethality: clearing up the misunderstandings. *Molecular Oncology* **5** 387–393. (doi:10.1016/j.molonc.2011.07.001)

Hennessy BT, Timms KM, Carey MS, Gutin A, Meyer LA, Flake DD 2nd, Abkevich V, Potter J, Pruss D, Glenn P, *et al.* 2010 Somatic mutations in BRCA1 and BRCA2 could expand the number of patients that benefit from poly (ADP ribose) polymerase inhibitors in ovarian cancer. *Journal of Clinical Oncology* **28** 3570–3576. (doi:10.1200/JCO.2009.27.2997)

Hernan I, Borras E, de Sousa Dias M, Gamundi MJ, Mane B, Llort G, Agundez JA, Blanca M & Carballo M 2012 Detection of genomic variations in BRCA1 and BRCA2 genes by long-range PCR and next-generation sequencing. *Journal of Molecular Diagnostics* **14** 286–293. (doi:10.1016/j.jmoldx.2012.01.013)

© 2016 Society for Endocrinology
Printed in Great Britain
Published by Bioscientifica Ltd.

Hilton JL, Geisler JP, Rathe JA, Hattermann-Zogg MA, DeYoung B & Buller RE 2002 Inactivation of BRCA1 and BRCA2 in ovarian cancer. *Journal of the National Cancer Institute* **94** 1396–1406. (doi:10.1093/jnci/94.18.1396)

Hoeijmakers JH 2001 Genome maintenance mechanisms for preventing cancer. *Nature* **411** 366–374. (doi:10.1038/35077232)

Hottiger MO, Hassa PO, Luscher B, Schuler H & Koch-Nolte F 2010 Toward a unified nomenclature for mammalian ADP-ribosyltransferases. *Trends in Biochemical Sciences* **35** 208–219. (doi:10.1016/j.tibs.2009.12.003)

Houtgraaf JH, Versmissen J & van der Giessen WJ 2006 A concise review of DNA damage checkpoints and repair in mammalian cells. *Cardiovascular Revascularization Medicine* **7** 165–172. (doi:10.1016/j.carrev.2006.02.002)

Hughes-Davies L, Huntsman D, Ruas M, Fuks F, Bye J, Chin SF, Milner J, Brown LA, Hsu F, Gilks B, *et al.* 2003 EMSY links the BRCA2 pathway to sporadic breast and ovarian cancer. *Cell* **115** 523–535. (doi:10.1016/S0092-8674(03)00930-9)

Ibragimova I & Cairns P 2011 Assays for hypermethylation of the BRCA1 gene promoter in tumor cells to predict sensitivity to PARP-inhibitor therapy. *Methods in Molecular Biology* **780** 277–291. (doi:10.1007/978-1-61779-270-0)

Ibrahim YH, Garcia-Garcia C, Serra V, He L, Torres-Lockhart K, Prat A, Anton P, Cozar P, Guzman M, Grueso J, *et al.* 2012 PI3K inhibition impairs BRCA1/2 expression and sensitizes BRCA-proficient triple-negative breast cancer to PARP inhibition. *Cancer Discovery* **2** 1036–1047. (doi:10.1158/2159-8290.CD-11-0348)

Jaspers JE, Kersbergen A, Boon U, Sol W, van Deemter L, Zander SA, Drost R, Wientjens E, Ji J, Aly A, *et al.* 2013 Loss of 53BP1 causes PARP inhibitor resistance in Brca1-mutated mouse mammary tumors. *Cancer Discovery* **3** 68–81. (doi:10.1158/2159-8290.CD-12-0049)

Ji J, Kinders RJ, Zhang Y, Rubinstein L, Kummar S, Parchment RE, Tomaszewski JE & Doroshow JH 2011 Modeling pharmacodynamic response to the poly(ADP-Ribose) polymerase inhibitor ABT-888 in human peripheral blood mononuclear cells. *PLoS ONE* **6** e26152. (doi:10.1371/journal.pone.0026152)

Johnson N, Johnson SF, Yao W, Li YC, Choi YE, Bernhardy AJ, Wang Y, Capelletti M, Sarosiek KA, Moreau LA, *et al.* 2013 Stabilization of mutant BRCA1 protein confers PARP inhibitor and platinum resistance. *PNAS* **110** 17041–17046. (doi:10.1073/pnas.1305170110)

Jones PA & Baylin SB 2002 The fundamental role of epigenetic events in cancer. *Nature Reviews. Genetics* **3** 415–428. (doi:10.1038/nrg816)

Joosse SA, van Beers EH, Tielen IH, Horlings H, Peterse JL, Hoogerbrugge N, Ligtenberg MJ, Wessels LF, Axwijk P, Verhoef S, *et al.* 2009 Prediction of BRCA1-association in hereditary non-BRCA1/2 breast carcinomas with array-CGH. *Breast Cancer Research and Treatment* **116** 479–489. (doi:10.1007/s10549-008-0117-z)

Kaufman B, Shapira-Frommer R, Schmutzler RK, Audeh MW, Friedlander M, Balmana J, Mitchell G, Fried G, Stemmer SM, Hubert A, *et al.* 2015 Olaparib monotherapy in patients with advanced cancer and a germline BRCA1/2 mutation. *Journal of Clinical Oncology* **33** 244–250. (doi:10.1200/JCO.2014.56.2728)

Kim G, Ison G, McKee AE, Zhang H, Tang S, Gwise T, Sridhara R, Lee E, Tzou A, Philip R, *et al.* 2015 FDA approval summary: olaparib monotherapy in patients with deleterious germline BRCA-mutated advanced ovarian cancer treated with three or more lines of chemotherapy. *Clinical Cancer Research* **21** 4257–4261. (doi:10.1158/1078-0432.CCR-15-0887)

Kinsella TJ 2009 Understanding DNA damage response and DNA repair pathways: applications to more targeted cancer therapeutics. *Seminars in Oncology* **36** S42–51. (doi:10.1053/j.seminoncol.2009.02.004)

Konstantinopoulos PA, Spentzos D, Karlan BY, Taniguchi T, Fountzilas E, Francoeur N, Levine DA & Cannistra SA 2010 Gene expression profile of BRCAness that correlates with responsiveness to chemotherapy and with outcome in patients with epithelial ovarian cancer. *Journal of Clinical Oncology* **28** 3555–3561. (doi:10.1200/JCO.2009.27.5719)

Larsen MJ, Kruse TA, Tan Q, Laenkholm AV, Bak M, Lykkesfeldt AE, Sorensen KP, Hansen TV, Ejlertsen B, Gerdes AM, *et al.* 2013 Classifications within molecular subtypes enables identification of BRCA1/BRCA2 mutation carriers by RNA tumor profiling. *PLoS ONE* **8** e64268. (doi:10.1371/journal.pone.0064268)

Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, Scott CL, Meier W, Shapira-Frommer R, Safra T, *et al.* 2014 Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: a preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. *Lancet Oncology* **15** 852–861. (doi:10.1016/S1470-2045(14)70228-1)

Leongamornlert D, Mahmud N, Tymrakiewicz M, Saunders E, Dadaev T, Castro E, Goh C, Govindasami K, Guy M, O'Brien L, *et al.* 2012 Germline BRCA1 mutations increase prostate cancer risk. *British Journal of Cancer* **106** 1697–1701. (doi:10.1038/bjc.2012.146)

Lesnock JL, Darcy KM, Tian C, Deloia JA, Thrall MM, Zahn C, Armstrong DK, Birrer MJ & Krivak TC 2013 BRCA1 expression and improved survival in ovarian cancer patients treated with intraperitoneal cisplatin and paclitaxel: a Gynecologic Oncology Group Study. *British Journal of Cancer* **108** 1231–1237. (doi:10.1038/bjc.2013.70)

Lips EH, Laddach N, Savola SP, Vollebergh MA, Oonk AM, Imholz AL, Wessels LF, Wesseling J, Nederlof PM & Rodenhuis S 2011a Quantitative copy number analysis by Multiplex Ligation-dependent Probe Amplification (MLPA) of BRCA1-associated breast cancer regions identifies BRCAness. *Breast Cancer Research* **13** R107. (doi:10.1186/bcr3049)

Lips EH, Mulder L, Hannemann J, Laddach N, Vrancken Peeters MT, van de Vijver MJ, Wesseling J, Nederlof PM & Rodenhuis S 2011b Indicators of homologous recombination deficiency in breast cancer and association with response to neoadjuvant chemotherapy. *Annals of Oncology* **22** 870–876. (doi:10.1093/annonc/mdq468)

Lord CJ & Ashworth A 2016 BRCAness revisited. *Nature Reviews. Cancer* **16** 110–120. (doi:10.1038/nrc.2015.21)

Loveday C, Turnbull C, Ramsay E, Hughes D, Ruark E, Frankum JR, Bowden G, Kalmyrzaev B, Warren-Perry M, Snape K, *et al.* 2011 Germline mutations in RAD51D confer susceptibility to ovarian cancer. *Nature Genetics* **43** 879–882. (doi:10.1038/ng.893)

Marquard AM, Eklund AC, Joshi T, Krzystanek M, Favero F, Wang ZC, Richardson AL, Silver DP, Szallasi Z & Birkbak NJ 2015 Pan-cancer analysis of genomic scar signatures associated with homologous recombination deficiency suggests novel indications for existing cancer drugs. *Biomarker Research* **3** 9. (doi:10.1186/s40364-015-0033-4)

Mateo J, Carreira S, Sandhu S, Miranda S, Mossop H, Perez-Lopez R, Nava Rodrigues D, Robinson D, Omlin A, Tunariu N, *et al.* 2015 DNA-repair defects and olaparib in metastatic prostate cancer. *New England Journal of Medicine* **373** 1697–1708. (doi:10.1056/NEJMoa1506859)

Matros E, Wang ZC, Lodeiro G, Miron A, Iglehart JD & Richardson AL 2005 BRCA1 promoter methylation in sporadic breast tumors: relationship to gene expression profiles. *Breast Cancer Research Treatment* **91** 179–186. (doi:10.1007/s10549-004-7603-8)

McNeish IA, Oza AM, Coleman RL, Scott CL, Konecny GE, Tinker A, O'Malley DM, Brenton J, Kristeleit RS, Bell-McGuinn K, *et al.* 2015 Results of ARIEL2: A Phase 2 trial to prospectively identify ovarian cancer patients likely to respond to rucaparib using tumor genetic analysis. *Journal of Clinical Oncology* **33** abstract 5508.

Meisel JL, Hyman DM, Garg K, Zhou Q, Dao F, Bisogna M, Gao J, Schultz ND, Grisham RN, Phillips M, *et al.* 2014 The performance of BRCA1 immunohistochemistry for detecting germline, somatic, and epigenetic BRCA1 loss in high-grade serous ovarian cancer. *Annals of Oncology* **25** 2372–2378. (doi:10.1093/annonc/mdu461)

Mendes-Pereira AM, Martin SA, Brough R, McCarthy A, Taylor JR, Kim JS, Waldman T, Lord CJ & Ashworth A 2009 Synthetic lethal targeting of PTEN mutant cells with PARP inhibitors. *EMBO Molecular Medicine* **1** 315–322. (doi:10.1002/emmm.200900041)

DOI: 10.1530/ERC-16-0116
© 2016 Society for Endocrinology
Printed in Great Britain
Published by Bioscientifica Ltd.

Michels J, Vitale I, Galluzzi L, Adam J, Olaussen KA, Kepp O, Senovilla L, Talhaoui I, Guegan J, Enot DP, *et al.* 2013 Cisplatin resistance associated with PARP hyperactivation. *Cancer Research* **73** 2271–2280. (doi:10.1158/0008-5472.CAN-12-3000)

Michels J, Vitale I, Saparbaev M, Castedo M & Kroemer G 2014 Predictive biomarkers for cancer therapy with PARP inhibitors. *Oncogene* **33** 3894–3907. (doi:10.1038/onc.2013.352)

Mukhopadhyay A, Elattar A, Cerbinskaite A, Wilkinson SJ, Drew Y, Kyle S, Los G, Hostomsky Z, Edmondson RJ & Curtin NJ 2010 Development of a functional assay for homologous recombination status in primary cultures of epithelial ovarian tumor and correlation with sensitivity to poly(ADP-ribose) polymerase inhibitors. *Clinical Cancer Research* **16** 2344–2351. (doi:10.1158/1078-0432.CCR-09-2758)

Murai J, Huang SY, Das BB, Renaud A, Zhang Y, Doroshow JH, Ji J, Takeda S & Pommier Y 2012 Trapping of PARP1 and PARP2 by clinical PARP inhibitors. *Cancer Research* **72** 5588–5599. (doi:10.1158/0008-5472.CAN-12-2753)

Norquist B, Wurz KA, Pennil CC, Garcia R, Gross J, Sakai W, Karlan BY, Taniguchi T & Swisher EM 2011 Secondary somatic mutations restoring BRCA1/2 predict chemotherapy resistance in hereditary ovarian carcinomas. *Journal of Clinical Oncology* **29** 3008–3015. (doi:10.1200/JCO.2010.34.2980)

O'Sullivan CC, Moon DH, Kohn EC & Lee JM 2014 Beyond breast and ovarian cancers: PARP inhibitors for BRCA mutation-associated and BRCA-like solid tumors. *Frontiers in Oncology* **4** 42. (doi:10.3389/fonc.2014.00042)

Oplustilova L, Wolanin K, Mistrik M, Korinkova G, Simkova D, Bouchal J, Lenobel R, Bartkova J, Lau A, O'Connor MJ, *et al.* 2012 Evaluation of candidate biomarkers to predict cancer cell sensitivity or resistance to PARP-1 inhibitor treatment. *Cell Cycle* **11** 3837–3850. (doi:10.4161/cc.22026)

Pal T, Permuth-Wey J, Betts JA, Krischer JP, Fiorica J, Arango H, LaPolla J, Hoffman M, Martino MA, Wakeley K, *et al.* 2005 BRCA1 and BRCA2 mutations account for a large proportion of ovarian carcinoma cases. *Cancer* **104** 2807–2816. (doi:10.1002/cncr.21536)

Patch AM, Christie EL, Etemadmoghadam D, Garsed DW, George J, Fereday S, Nones K, Cowin P, Alsop K, Bailey PJ, *et al.* 2015 Whole-genome characterization of chemoresistant ovarian cancer. *Nature* **521** 489–494. (doi:10.1038/nature14410)

Paul I, Savage KI, Blayney JK, Lamers E, Gately K, Kerr K, Sheaff M, Arthur K, Richard DJ, Hamilton PW, *et al.* 2011 PARP inhibition induces BAX/BAK-independent synthetic lethality of BRCA1-deficient non-small cell lung cancer. *Journal of Pathology* **224** 564–574. (doi:10.1002/path.2925)

Peng G, Chun-Jen Lin C, Mo W, Dai H, Park YY, Kim SM, Peng Y, Mo Q, Siwko S, Hu R, *et al.* 2014 Genome-wide transcriptome profiling of homologous recombination DNA repair. *Nature Communications* **5** 3361.

Pennington KP, Walsh T, Harrell MI, Lee MK, Pennil CC, Rendi MH, Thornton A, Norquist BM, Casadei S, Nord AS, *et al.* 2014 Germline and somatic mutations in homologous recombination genes predict platinum response and survival in ovarian, fallopian tube, and peritoneal carcinomas. *Clinical Cancer Research* **20** 764–775. (doi:10.1158/1078-0432.CCR-13-2287)

Polo SE & Jackson SP 2011 Dynamics of DNA damage response proteins at DNA breaks: a focus on protein modifications. *Genes & Development* **25** 409–433.

Rebbeck TR, Mitra N, Wan F, Sinilnikova OM, Healey S, McGuffog L, Mazoyer S, Chenevix-Trench G, Easton DF, Antoniou AC, *et al.* 2015 Association of type and location of BRCA1 and BRCA2 mutations with risk of breast and ovarian cancer. *JAMA* **313** 1347–1361.

Redon CE, Nakamura AJ, Zhang YW, Ji JJ, Bonner WM, Kinders RJ, Parchment RE, Doroshow JH & Pommier Y 2010 Histone gammaH2AX and poly(ADP-ribose) as clinical pharmacodynamic biomarkers. *Clinical Cancer Research* **16** 4532–4542. (doi:10.1158/1078-0432.CCR-10-0523)

Rice JC, Ozcelik H, Maxeiner P, Andrulis I & Futscher BW 2000 Methylation of the BRCA1 promoter is associated with decreased BRCA1 mRNA levels in clinical breast cancer specimens. *Carcinogenesis* **21** 1761–1765. (doi:10.1093/carcin/21.9.1761)

Rigakos G & Razis E 2012 BRCAness: finding the Achilles heel in ovarian cancer. *Oncologist* **17** 956–962. (doi:10.1634/theoncologist.2012-0028)

Robinson D, Van Allen EM, Wu YM, Schultz N, Lonigro RJ, Mosquera JM, Montgomery B, Taplin ME, Pritchard CC, Attard G, *et al.* 2015 Integrative clinical genomics of advanced prostate cancer. *Cell* **161** 1215–1228.

Rouleau M, Patel A, Hendzel MJ, Kaufmann SH & Poirier GG 2010 PARP inhibition: PARP1 and beyond. *Nature Reviews. Cancer* **10** 293–301. (doi:10.1038/nrc2812)

Ruiz A, Llort G, Yague C, Baena N, Vinas M, Torra M, Brunet A, Segui MA, Saigi E & Guitart M 2014 Genetic testing in hereditary breast and ovarian cancer using massive parallel sequencing. *BioMed Research International* **2014** 542541. (doi:10.1155/2014/542541)

Ruscito I, Dimitrova D, Vasconcelos I, Gellhaus K, Schwachula T, Bellati F, Zeillinger R, Benedetti-Panici P, Vergote I, Mahner S, *et al.* 2014 BRCA1 gene promoter methylation status in high-grade serous ovarian cancer patients – a study of the tumour Bank ovarian cancer (TOC) and ovarian cancer diagnosis consortium (OVCAD). *European Journal of Cancer* **50** 2090–2098. (doi:10.1016/j.ejca.2014.05.001)

Sakai W, Swisher EM, Karlan BY, Agarwal MK, Higgins J, Friedman C, Villegas E, Jacquemont C, Farrugia DJ, Couch FJ, *et al.* 2008 Secondary mutations as a mechanism of cisplatin resistance in BRCA2-mutated cancers. *Nature* **451** 1116–1120. (doi:10.1038/nature06633)

Sakai W, Swisher EM, Jacquemont C, Chandramohan KV, Couch FJ, Langdon SP, Wurz K, Higgins J, Villegas E & Taniguchi T 2009 Functional restoration of BRCA2 protein by secondary BRCA2 mutations in BRCA2-mutated ovarian carcinoma. *Cancer Research* **69** 6381–6386. (doi:10.1158/0008-5472.CAN-09-1178)

Savage KI, Gorski JJ, Barros EM, Irwin GW, Manti L, Powell AJ, Pellagatti A, Lukashchuk N, McCance DJ, McCluggage WG, *et al.* 2014 Identification of a BRCA1-mRNA splicing complex required for efficient DNA repair and maintenance of genomic stability. *Molecular Cell* **54** 445–459. (doi:10.1016/j.molcel.2014.03.021)

Schelman WR, Sandhu SK, Moreno Garcia V, Wilding G, Sun L, Toniatti C, Stroh M, Kreischer N, Carpenter CL, Molife LR, *et al.* 2011 First-in human trial of poly(ADP)-ribose polymerase (PARP) inhibitor MK-4827 in advanced cancer patients with anti- tumor activity in BRCA-deficient tumors and sporadic ovarian cancers *Journal of Clinical Oncology* **29** abstract 3102.

Schreiber V, Dantzer F, Ame JC & de Murcia G 2006 Poly(ADP-ribose): novel functions for an old molecule. *Nature Reviews: Molecular Cell Biology* **7** 517–528. (doi:10.1038/nrm1963)

Sharrocks AD 2001 The ETS-domain transcription factor family. *Nature Reviews. Molecular Cell Biology* **2** 827–837. (doi:10.1038/35099076)

Shen WH, Balajee AS, Wang J, Wu H, Eng C, Pandolfi PP & Yin Y 2007 Essential role for nuclear PTEN in maintaining chromosomal integrity. *Cell* **128** 157–170. (doi:10.1016/j.cell.2006.11.042)

Sims D, Sudbery I, Ilott NE, Heger A & Ponting CP 2014 Sequencing depth and coverage: key considerations in genomic analyses. *Nature Reviews: Genetics* **15** 121–132. (doi:10.1038/nrg3642)

Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, Deng S, Johnsen H, Pesich R, Geisler S, *et al.* 2003 Repeated observation of breast tumor subtypes in independent gene expression data sets. *PNAS* **100** 8418–8423. (doi:10.1073/pnas.0932692100)

Stefansson OA, Jonasson JG, Johannsson OT, Olafsdottir K, Steinarsdottir M, Valgeirsdottir S & Eyfjord JE 2009 Genomic profiling of breast tumours in relation to BRCA abnormalities and phenotypes. *Breast Cancer Research* **11** R47.

© 2016 Society for Endocrinology
Printed in Great Britain
Published by Bioscientifica Ltd.

Strom CE & Helleday T 2012 Strategies for the use of poly(adenosine diphosphate ribose) polymerase (PARP) inhibitors in cancer therapy. *Biomolecules* **2** 635–649. (doi:10.3390/biom2040635)

Tan DS, Rothermundt C, Thomas K, Bancroft E, Eeles R, Shanley S, Ardern-Jones A, Norman A, Kaye SB & Gore ME 2008 "BRCAness" syndrome in ovarian cancer: a case-control study describing the clinical features and outcome of patients with epithelial ovarian cancer associated with BRCA1 and BRCA2 mutations. *Journal of Clinical Oncology* **26** 5530–5536. (doi:10.1200/JCO.2008.16.1703)

Tan SH, Furusato B, Fang X, He F, Mohamed AA, Griner NB, Sood K, Saxena S, Katta S, Young D, *et al.* 2014 Evaluation of ERG responsive proteome in prostate cancer. *Prostate* **74** 70–89. (doi:10.1002/pros.22731)

Taniguchi T, Tischkowitz M, Ameziane N, Hodgson SV, Mathew CG, Joenje H, Mok SC & D'Andrea AD 2003 Disruption of the Fanconi anemia-BRCA pathway in cisplatin-sensitive ovarian tumors. *Nature Medicine* **9** 568–574. (doi:10.1038/nm852)

Telli ML, Timms KM, Reid JE, Hennessy B, Mills GB, Jensen KC, Szallasi Z, Barry WT, Winer EP, Tung N, *et al.* 2016 Homologous recombination deficiency (HRD) score predicts response to platinum-containing neoadjuvant chemotherapy in patients with triple negative breast cancer. *Clinical Cancer Research* [in press].

Teodoridis JM, Hall J, Marsh S, Kannall HD, Smyth C, Curto J, Siddiqui N, Gabra H, McLeod HL, Strathdee G, *et al.* 2005 CpG island methylation of DNA damage response genes in advanced ovarian cancer. *Cancer Research* **65** 8961–8967. (doi:10.1158/0008-5472.CAN-05-1187)

Timms KM, Abkevich V, Hughes E, Neff C, Reid J, Morris B, Kalva S, Potter J, Tran TV, Chen J, *et al.* 2014 Association of BRCA1/2 defects with genomic scores predictive of DNA damage repair deficiency among breast cancer subtypes. *Breast Cancer Research* **16** 475.

Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, Varambally S, Cao X, Tchinda J, Kuefer R, *et al.* 2005 Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. *Science* **310** 644–648. (doi:10.1126/science.1117679)

Turner NC & Ashworth A 2011 Biomarkers of PARP inhibitor sensitivity. *Breast Cancer Research and Treatment* **127** 283–286. (doi:10.1007/s10549-011-1375-8)

Turner N, Tutt A & Ashworth A 2004 Hallmarks of 'BRCAness' in sporadic cancers. *Nature Reviews. Cancer* **4** 814–819. (doi:10.1038/nrc1457)

Tutt A & Ashworth A 2002 The relationship between the roles of BRCA genes in DNA repair and cancer predisposition. *Trends in Molecular Medicine* **8** 571–576. (doi:10.1016/S1471-4914(02)02434-6)

Tutt A, Bertwistle D, Valentine J, Gabriel A, Swift S, Ross G, Griffin C, Thacker J & Ashworth A 2001 Mutation in Brca2 stimulates error-prone homology-directed repair of DNA double-strand breaks occurring between repeated sequences. *EMBO Journal* **20** 4704–4716. (doi:10.1093/emboj/20.17.4704)

Tutt A, Robson M, Garber JE, Domchek SM, Audeh MW, Weitzel JN, Friedlander M, Arun B, Loman N, Schmutzler RK, *et al.* 2010 Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: a proof-of-concept trial. *Lancet* **376** 235–244. (doi:10.1016/S0140-6736(10)60892-6)

Vaz FH, Machado PM, Brandao RD, Laranjeira CT, Eugenio JS, Fernandes AH & Andre SP 2007 Familial breast/ovarian cancer and BRCA1/2 genetic screening: the role of immunohistochemistry as an additional method in the selection of patients. *Journal of Histochemistry and Cytochemistry* **55** 1105–1113. (doi:10.1369/jhc.7A7209.2007)

Venkitaraman AR 2002 Cancer susceptibility and the functions of BRCA1 and BRCA2. *Cell* **108** 171–182. (doi:10.1016/S0092-8674(02)00615-3)

Venkitaraman AR 2003 A growing network of cancer-susceptibility genes. *New England Journal of Medicine* **348** 1917–1919. (doi:10.1056/NEJMcibr023150)

Vollebergh MA, Lips EH, Nederlof PM, Wessels LF, Schmidt MK, van Beers EH, Cornelissen S, Holtkamp M, Froklage FE, de Vries EG, *et al.* 2011 An aCGH classifier derived from BRCA1-mutated breast cancer and benefit of high-dose platinum-based chemotherapy in HER2-negative breast cancer patients. *Annals of Oncology* **22** 1561–1570. (doi:10.1093/annonc/mdq624)

Vollebergh MA, Lips EH, Nederlof PM, Wessels LF, Wesseling J, Vd Vijver MJ, de Vries EG, van Tinteren H, Jonkers J, Hauptmann M, *et al.* 2014 Genomic patterns resembling BRCA1- and BRCA2-mutated breast cancers predict benefit of intensified carboplatin-based chemotherapy. *Breast Cancer Research* **16** R47.

Walsh T, Casadei S, Lee MK, Pennil CC, Nord AS, Thornton AM, Roeb W, Agnew KJ, Stray SM, Wickramanayake A, *et al.* 2011 Mutations in 12 genes for inherited ovarian, fallopian tube, and peritoneal carcinoma identified by massively parallel sequencing. *PNAS* **108** 18032–18037. (doi:10.1073/pnas.1115052108)

Wang M, Wu W, Wu W, Rosidi B, Zhang L, Wang H & Iliakis G 2006 PARP-1 and Ku compete for repair of DNA double strand breaks by distinct NHEJ pathways. *Nucleic Acids Research* **34** 6170–6182. (doi:10.1093/nar/gkl840)

Wang ZC, Birkbak NJ, Culhane AC, Drapkin R, Fatima A, Tian R, Schwede M, Alsop K, Daniels KE, Piao H, *et al.* 2012 Profiles of genomic instability in high-grade serous ovarian cancer predict treatment outcome. *Clinical Cancer Research* **18** 5806–5815. (doi:10.1158/1078-0432.CCR-12-0857)

Watkins JA, Irshad S, Grigoriadis A & Tutt AN 2014 Genomic scars as biomarkers of homologous recombination deficiency and drug response in breast and ovarian cancers. *Breast Cancer Research* **16** 211. (doi:10.1186/bcr3670)

Watkins J, Weekes D, Shah V, Gazinska P, Joshi S, Sidhu B, Gillett C, Pinder S, Vanoli F, Jasin M, *et al.* 2015 Genomic complexity profiling reveals that HORMAD1 overexpression contributes to homologous recombination deficiency in triple-negative breast cancers. *Cancer Discovery* **5** 488–505. (doi:10.1158/2159-8290.CD-14-1092)

Weaver AN & Yang ES 2013 Beyond DNA repair: additional functions of PARP-1 in cancer. *Frontiers in Oncology* **3** 290.

Wilcoxen KM, Becker M, Neff C, Abkevich V, Jones JT, Hou X, Wang Y, Hartman AR, AlHilli MM, Gutin A, *et al.* 2015 Use of homologous recombination deficiency (HRD) score to enrich for niraparib sensitive high grade ovarian tumors. *Journal of Clinical Oncology* **33** 5532.

Willers H, Taghian AG, Luo CM, Treszezamsky A, Sgroi DC & Powell SN 2009 Utility of DNA repair protein foci for the detection of putative BRCA1 pathway defects in breast cancer biopsies. *Molecular Cancer Research* **7** 1304–1309. (doi:10.1158/1541-7786.MCR-09-0149)

Wu L 2008 Wrestling off RAD51: a novel role for RecQ helicases. *Bioessays* **30** 291–295. (doi:10.1002/bies.20735)

Xia F, Taghian DG, DeFrank JS, Zeng ZC, Willers H, Iliakis G & Powell SN 2001 Deficiency of human BRCA2 leads to impaired homologous recombination but maintains normal nonhomologous end joining. *PNAS* **98** 8644–8649. (doi:10.1073/pnas.151253498)

Xu G, Chapman JR, Brandsma I, Yuan J, Mistrik M, Bouwman P, Bartkova J, Gogola E, Warmerdam D, Barazas M, *et al.* 2015 REV7 counteracts DNA double-strand break resection and affects PARP inhibition. *Nature* **521** 541–544. (doi:10.1038/nature14328)

Yap TA, Sandhu SK, Carden CP & de Bono JS 2011 Poly(ADP-ribose) polymerase (PARP) inhibitors: exploiting a synthetic lethal strategy in the clinic. *CA: A Cancer Journal for Clinicians* **61** 31–49. (doi:10.3322/caac.20095)

Received in final form 22 May 2016
Accepted 23 May 2016
Accepted Preprint published online 25 May 2016

© 2016 Society for Endocrinology
Printed in Great Britain